(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(72) Inventors: LASKEN, Roger, S.; 364 Central Avenue,
First Floor, New Haven, CT 06515 (US). EGHOLM,
Michael; 8 Dogwood Court, Woodbridge, CT 06525 (US).
ALSMADI, Osama, A.; 310 Glenwood Drive, Guilford,
CT 06437 (US). BORNARTH, Carole; 99 Mohawk
Drive, Wallingford, CT 06492 (US). WISNIEWSKI,
Michele; 8302 Town Walk Drive, Hamden, CT 06518
(US). HOSONO, Seiyu; 140 New Street, Apt. 2315,
Mamaroneck, NY 10543 (US). RAGHUNATHAN, Aru-
mugham; 80 Brentwood Drive, Cheshire, CT 06410 (US).

(74) Agents: NEEDLE, William, H. et al.; Needle & Rosen-
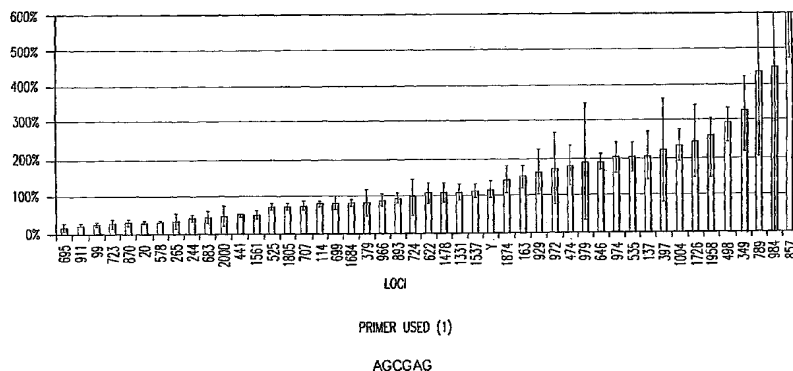berg, P.C., Suite 1000, 999 Peachtree Street, Atlanta, GA
30309-3915 (US).

(54) Title: NUCLEIC ACID AMPLIFICATION



SUMMARY OF 47 LOCI REPRESENTATION WITH A SINGLE PRIMER (AluR 11)

PRIMER USED (1)

AGCGAG

(57) Abstract: Disclosed are compositions and a method for amplification of nucleic acid sequences of interest. The method is based on strand displacement replication of the nucleic acid sequences by primers. The disclosed method is a form of multiple displacement amplification (MDA) useful for amplifying genomic nucleic acid samples and other nucleic acid samples of high complexity. The disclosed method can be used to amplify such highly complex nucleic acid samples using only one or a limited number of primers. It has been discovered that one or a small number of primers can effectively amplify whole genomes an other nucleic acid samples of high sequence complexity. The primers are specially selected or designed to be able to prime and efficiently amplify the broad range of sequences present in highly complex nucleic acid samples despite the limited amount of primer sequence represented in the primers. It has been discovered that generation of high molecular weight artifacts, in an isothermal amplification procedure, is substantially reduced or eliminated while still allowing the desired amplification of input DNA by carrying out the reaction at a higher temperature and, optionally, in the presence of one or more additives. It has also bee discovered that amplification reactions can produce amplification products of high quality, such as low amplification bias, if performed on an amount of nucleic acid at or over a threshold amount and/or on nucleic acids at or below a threshold concentration.

**Published:**
— *without international search report and to be republished upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# NUCLEIC ACID AMPLIFICATION

## FIELD OF THE INVENTION

The disclosed invention is generally in the field of nucleic acid amplification.

## BACKGROUND OF THE INVENTION

5       A number of methods have been developed for exponential amplification of nucleic acids. These include the polymerase chain reaction (PCR), ligase chain reaction (LCR), self-sustained sequence replication (3SR), nucleic acid sequence based amplification (NASBA), strand displacement amplification (SDA), and amplification with Qβ replicase (Birkenmeyer and Mushahwar, *J. Virological Methods*, 35:117-126 (1991); Landegren,

10      *Trends Genetics* 9:199-202 (1993)).

Fundamental to most genetic analysis is availability of genomic DNA of adequate quality and quantity. Since DNA yield from human samples is frequently limiting, much effort has been invested in general methods for propagating and archiving genomic DNA. Methods include the creation of EBV-transformed cell lines or whole genome amplification

15      (WGA) by random or degenerate oligonucleotide-primed PCR. Whole genome PCR, a variant of PCR amplification, involves the use of random or partially random primers to amplify the entire genome of an organism in the same PCR reaction. This technique relies on having a sufficient number of primers of random or partially random sequence such that pairs of primers will hybridize throughout the genomic DNA at moderate intervals.

20      Replication initiated at the primers can then result in replicated strands overlapping sites where another primer can hybridize. By subjecting the genomic sample to multiple amplification cycles, the genomic sequences will be amplified. Whole genome PCR has the same disadvantages as other forms of PCR. However, WGA methods suffer from high cost or insufficient coverage and inadequate average DNA size (Telenius *et al.*, *Genomics.*

25      13:718-725 (1992); Cheung and Nelson, *Proc Natl Acad Sci U S A.* 93:14676-14679 (1996); Zhang *et al.*, *Proc Natl Acad Sci U S A.* 89:5847-5851 (1992)).

Another field in which amplification is relevant is RNA expression profiling, where the objective is to determine the relative concentration of many different molecular species of RNA in a biological sample. Some of the RNAs of interest are present in relatively low

30      concentrations, and it is desirable to amplify them prior to analysis. It is not possible to use the polymerase chain reaction to amplify them because the mRNA mixture is complex, typically consisting of 5,000 to 20,000 different molecular species. The polymerase chain

reaction has the disadvantage that different molecular species will be amplified at different rates, distorting the relative concentrations of mRNAs.

       Some procedures have been described that permit moderate amplification of all RNAs in a sample simultaneously. For example, in Lockhart *et al.*, *Nature Biotechnology*

5     14:1675-1680 (1996), double-stranded cDNA was synthesized in such a manner that a strong RNA polymerase promoter was incorporated at the end of each cDNA. This promoter sequence was then used to transcribe the cDNAs, generating approximately 100 to 150 RNA copies for each cDNA molecule. This weak amplification system allowed RNA profiling of biological samples that contained a minimum of 100,000 cells. However, there

10    is a need for a more powerful amplification method that would permit the profiling analysis of samples containing a very small number of cells.

       Another form of nucleic acid amplification, involving strand displacement, has been described in U.S. Patent No. 6,124,120 to Lizardi. In one form of the method, two sets of primers are used that are complementary to opposite strands of nucleotide sequences

15    flanking a target sequence. Amplification proceeds by replication initiated at each primer and continuing through the target nucleic acid sequence, with the growing strands encountering and displacing previously replicated strands. In another form of the method a random set of primers is used to randomly prime a sample of genomic nucleic acid. The primers in the set are collectively, and randomly, complementary to nucleic acid sequences

20    distributed throughout nucleic acid in the sample. Amplification proceeds by replication initiating at each primer and continuing so that the growing strands encounter and displace adjacent replicated strands. In another form of the method concatenated DNA is amplified by strand displacement synthesis with either a random set of primers or primers complementary to linker sequences between the concatenated DNA. Synthesis proceeds

25    from the linkers, through a section of the concatenated DNA to the next linker, and continues beyond, with the growing strands encountering and displacing previously replicated strands.

       MDA of genomic DNA or circularized bacterial or plasmid DNA can be carried out using random primers at a temperature which is optimal for the DNA polymerase.

30    Generally, this is in a lower temperature range, such as 30-34°C. The DNA to be amplified can be referred to as, for example, the target sequence, template, specific template, input DNA, template DNA, and specific input DNA. The goal of MDA is to amply this input DNA. However, DNA polymerase also can produce undesirable artifacts during these

MDA reactions. Such artifacts produced by DNA polymerase and random primers at temperatures that are optimal for the DNA polymerase activity are also observed in other amplification techniques, such as rolling circle amplification (RCA) (examples include multiply-primed RCA, multiply-primed RCA of circular DNA circularized cDNA in

5      isothermal total transcript amplification (ITTA) and multiply-primed RCA of circularized dsDNA using random hexamer and sequence specific primers). The defining characteristic of the artifact DNA is that it does not represent the specific sequence of the input DNA. In fact, the artifact DNA can be produced in the absence of any input DNA (as in the case of control reactions). This is problematic since control reactions lacking input DNA are often

10     carried out with the expectation that no product DNA will be synthesized. Therefore, it would be advantageous to reduce or eliminate the artifact synthesis. Artifact DNA is generally of high molecular weight and therefore is often indistinguishable from the desired specific amplified DNA based on size. In the case where specific input DNA is present, the artifact DNA product can be generated and interferes with the desired use of the specific

15     DNA product, and again, reduction or elimination of artifact production would be beneficial.

           In contrast to the low molecular weight artifacts generated during polymerase chain reaction (PCR), referred to as primer dimers, the MDA artifacts are of high molecular weight with lengths ranging from several hundred basepairs to greater than 20 kb. Similar

20     high molecular weight artifacts have also been described for other isothermal amplification systems such as ERCA (PCT/AU99/01110 (Hafner)). Contaminating nucleic acids in DNA polymerase preparations are one of the possible source of undesired template for the generation of these artifacts.

## BRIEF SUMMARY OF THE INVENTION

25             Disclosed are compositions and a method for amplification of nucleic acid sequences of interest. The method is based on strand displacement replication of the nucleic acid sequences by primers. The disclosed method is a form of multiple displacement amplification (MDA) useful for amplifying genomic nucleic acid samples and other nucleic acid samples of high complexity. The disclosed method can be used to amplify such highly

30     complex nucleic acid samples using only one or a limited number of primers. It has been discovered that one or a small number of primers can effectively amplify whole genomes and other nucleic acid samples of high sequence complexity. The primers are specially selected or designed to be able to prime and efficiently amplify the broad range of

sequences present in highly complex nucleic acid samples despite the limited amount of primer sequence represented in the primers. The disclosed method generally involves bringing into contact one, a few, or more primers having specific nucleic acid sequences, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under

5      conditions that promote replication of nucleic acid molecules in the nucleic acid sample. Replication of the nucleic acid molecules results in replicated strands such that, during replication, the replicated strands are displaced from the nucleic acid molecules by strand displacement replication of another replicated strand. The replication can result in amplification of all or a substantial fraction of the nucleic acid molecules in the nucleic acid

10     sample. In one form of the disclosed method, which is a form of whole genome strand displacement amplification (WGSDA), one, a few, or more primers are used to prime a sample of genomic nucleic acid (or another sample of nucleic acid of high complexity).

It was discovered that highly complex nucleic acid samples can be efficiently amplified using only one or a few primers having specific nucleic acid sequences. The one

15     or few primers are complementary to nucleic acid sequences distributed throughout nucleic acid molecules in the sample. For example, a single 6 base primer will be complementary to a sequence once every 4096 nucleotides, on average, and two 6 base primers collectively will be complementary to a sequence once every 2048 nucleotides, on average. It was discovered that such distributions of priming sites were sufficient to allow efficient multiple

20     displacement amplification. It was also discovered that such distributions of priming sites result in amplification of nucleic acid samples with broad coverage of the sequences in the nucleic acid samples and in amplification products with high sequence and locus representation and low amplification bias. Thus, the disclosed method can result in replication of all or a substantial fraction of the nucleic acid molecules in a nucleic acid

25     sample.

Amplification in the disclosed method proceeds by replication with a highly processive polymerase initiating at each primer and continuing until spontaneous termination. A key feature of the method is that as a DNA polymerase extends a primer, the polymerase displaces the replication products (that is, DNA strands) that resulted from

30     extension of other primers. The polymerase is continuously extending new primers and displacing the replication products of previous priming events. In this way, multiple overlapping copies of all of the nucleic acid molecules and sequences in the sample (for example, an entire genome) can be synthesized in a short time. The method has advantages

over prior amplification methods in that many fewer primers can be used. Further, the primers need not have a sequence specific for a given nucleic acid sample. Rather, the same primer or primers can be used to amplify a nucleic acid sample having unknown sequence. For example, a single primer as disclosed herein can be used to efficiently amplify any

5      whole genome from any source, an entire cosmid library, artificial chromosomes, and so on, all without the need to know any sequence present in the sample.

The disclosed method can accurately and evenly amplify the various sequences in highly complex nucleic acid samples. This result can be quantified by references to, for example sequence representation, locus representation and amplification bias. For example,

10     the replicated nucleic acid molecules produced in the disclosed method can have a sequence representation of at least 50% for at least 10 different target sequences. The amplification bias can be less than 10% for at least 10 different target sequences.

The method has advantages over the polymerase chain reaction since it can be carried out under isothermal conditions. Other advantages of whole genome strand

15     displacement amplification include a higher level of amplification than whole genome PCR (up to five times higher), amplification is less sequence-dependent than PCR, and there are no re-annealing artifacts or gene shuffling artifacts as can occur with PCR (since there are no cycles of denaturation and re-annealing).

In some useful embodiments of WGSDA, the nucleic acid sample is not subjected to

20     denaturing conditions, the primers are hexamer primers and contain modified nucleotides such that the primers are nuclease resistant, the DNA polymerase is φ29 DNA polymerase, or any combination of these features. The genome can be any type of genome, such as a microbial genome, a viral genome, a eukayotic genome, a plant genome, an animal genome, a vertebrate genome, a mammalian genome, or a human genome.

25     In one embodiment of the disclosed method, the nucleic acid sample is not subjected to denaturing conditions. Nucleic acid molecules, genomic DNA, for example, need not be denatured for efficient multiple displacement amplification. Elimination of a denaturation step and denaturation conditions has additional advantages such as reducing sequence bias in the amplified products.

30     In another embodiment, the primers can be, for example, at least 8 bases long, at least 7 bases long, at least 6 bases long, 5 bases long, 4 bases long, at least 3 bases long, or at least 2 bases long. Such short primers can still prime multiple strand displacement replication efficiently. Such short primers are easier and less expensive to produce. The

primers can have any sequence or can have particular sequences. For example, shorter primers, such as 6 nucleotide primers, will have complements in the nucleic acid sample at sufficiently short intervals to allow efficient and even amplification. Longer primers for use in the disclosed method generally will benefit from having sequences that are

5    complementary to specific sequences that occur at intervals throughout the nucleic acid sample. For example, the primers can be complementary to sequence in a repeat sequence, such as a microsatellite sequence, a minisatellite sequence, a satellite sequence, a transposon sequence, a ribosomal RNA sequence, a short interspersed nuclear element (SINE), or a long interspersed nuclear element (LINE); a functional consensus sequence such as a

10   promoter sequence, an enhancer sequence, a silencer sequence, an upstream regulatory element sequence, a transcription termination site sequence, a transposon regulatory sequence, a ribosomal RNA regulatory sequence, or a polyadenylation site sequence. Shorter primers can also includes such repeated sequences. When using repeated sequences in primers, more primers can be used in the reaction to improve the distribution of primer

15   complement sequences in the nucleic acid sample. In particular, where some or all of the repeated sequences have uneven distributions in the nucleic acids of the nucleic acid sample, multiple primers complementary to different repeated sequences can be used.

In another embodiment, the primers can each contain at least one modified nucleotide such that the primers are nuclease resistant. In another embodiment, the primers

20   can each contain at least one modified nucleotide such that the melting temperature of the primer is altered relative to a primer of the same sequence without the modified nucleotide(s). For these last two embodiments, it is preferred that the primers are modified RNA. In another embodiment, the DNA polymerase can be ϕ29 DNA polymerase, or another suitable DNA polymerase. ϕ29 DNA polymerase produces greater amplification in

25   multiple displacement amplification. The combination of two or more of the above features also yields improved results in multiple displacement amplification. In a preferred embodiment, for example, the nucleic acid sample is not subjected to denaturing conditions, the primers are 6 base primers and contain modified nucleotides such that the primers are nuclease resistant, and the DNA polymerase is ϕ29 DNA polymerase. The above features

30   are especially useful in whole genome strand displacement amplification (WGSDA).

Also disclosed are compositions and methods for amplification of nucleic acid sequences of interest with greater efficiency and fidelity. The disclosed method relates to isothermal amplification techniques, such as Multiple Displacement Amplification (MDA),

6

where the generation of DNA artifacts is decreased or eliminated. Generally, this can be accomplished by carrying out the reaction at elevated temperature. In particularly useful embodiments of the method, sugars and/or other additives can be used to stabilized the polymerase at high temperature.

5         It has been discovered that generation of high molecular weight artifacts, in an isothermal amplification procedure, is substantially reduced or eliminated while still allowing the desired amplification of input DNA by carrying out the reaction at a higher temperature and, optionally, in the presence of one or more additives. For example, the amplification reaction can be carried out in the presence of sugars at a temperature that is

10        higher then the normal optimal temperature for the DNA polymerase being used. It also has been discovered that isothermal amplification reactions can produce amplification products of high quality, such as low amplification bias, if performed at a higher temperature and, optionally, in the presence of one or more additives.

        The disclosed method fundamentally differs from techniques designed to eliminate

15        the generation of primer dimer artifacts in PCR. In the case of PCR, an increase in elongation or primer annealing temperature produces less primer dimers relative to priming of specific input DNA template. In contrast, artifacts addressed by the disclosed method are believed to be derived from minute amounts of contaminating DNA, such as plasmid cloning vectors known to be present in recombinant proteins, such as DNA polymerases, or

20        from trace contaminating DNA present in typical molecular biology laboratories in aerosol form or on equipment or in reagents. This complicates efforts to distinguish contaminant template from specific input template. It has been discovered that artifactual DNA synthesis can be reduced or eliminated by performing isothermal amplification reactions, such as MDA reactions, at elevated reaction temperatures, such that amplification of the

25        specific input template is favored over amplification of contaminating template.

        As an illustrative example, MDA of genomic DNA or circularized bacterial or plasmid DNA can be carried out using random hexamer primers and Phi29 DNA polymerase at a temperature which is optimal for Phi29 DNA polymerase activity (30-34°C). DNA templates (input DNA), such as genomic DNA, are added to the reaction for

30        amplification. However, Phi29 DNA polymerase also can produce undesirable artifacts during these MDA reactions. Generation of such artifacts by Phi29 DNA polymerase and random hexamer primers at temperatures that are optimal for Phi29 DNA polymerase activity (30-34°C) are also observed in other isothermal amplification reactions, such as

multiply-primed RCA of circularized cDNA in isothermal total transcript amplification (ITTA) and multiply-primed RCA of circularized dsDNA using random hexamer and sequence specific primers. The disclosed method and compositions can be used in MDA reactions with hexamer primers and Phi29 DNA polymerase to produce amplification

5 products with reduced or undetectable levels of artifactual DNA.

Some forms of the methods are based on strand displacement replication of the nucleic acid sequences by multiple primers. Such forms of the disclosed method generally involve incubating nucleic acids comprising target sequences at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity,

10 an additive, and a set of primers, under conditions promoting replication of the nucleic acids. Replication of the nucleic acids results in replicated strands. During replication at least one of the replicated nucleic acid strands is displaced by strand displacement replication of another replicated strand. Formation of replicated strands from the target sequences is favored over formation of replicated strands from non-target sequences.

15 Also disclosed is a method of amplifying nucleic acids, the method comprising incubating nucleic acids comprising target sequences at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, and a set of primers, under conditions promoting replication of the nucleic acids. Replication of the nucleic acids results in replicated strands. During replication at least one

20 of the replicated nucleic acid strands is displaced by strand displacement replication of another replicated strand. Formation of replicated strands from the target sequences is favored over formation of replicated strands from non-target sequences.

Also disclosed is a method of amplifying a whole genome, the method comprising exposing cells to alkaline conditions to form a cell lysate, reducing the pH of the cell lysate

25 to form a stabilized cell lysate, and incubating stabilized cell lysate at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, and a set of primers, under conditions promoting replication of the nucleic acids. Replication of the nucleic acids results in replicated strands. During replication at least one of the replicated nucleic acid strands is displaced by

30 strand displacement replication of another replicated strand. Formation of replicated strands from the target sequence is favored over formation of replicated strands from non-target sequences. The cell lysate comprises a whole genome.

Also disclosed is a method of performing strand displacement nucleic acid synthesis at an elevated temperature, the method comprising mixing thermolabile nucleic acid polymerase having strand-displacement activity, nucleic acids comprising target sequences, a set of primers, and an additive, and incubating at an elevated temperature and under

5      conditions favoring hybridization of the primers to the target sequences and extension of the primers by the addition of nucleotides sequentially to the 3' end of the primer in a template-dependent manner, wherein the extension results in replication of the target sequences.

Also disclosed is a method of amplifying a whole genome, the method comprising exposing cells to alkaline conditions to form a cell lysate, wherein the cell lysate comprises

10     a whole genome, reducing the pH of the cell lysate to form a stabilized cell lysate, and incubating stabilized cell lysate at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, a set of primers, and deoxyribonucleotide triphosphates under conditions promoting replication of nucleic acids. During replication at least one of the replicated nucleic acid strands is displaced by

15     strand displacement replication of another replicated strand. Formation of template-dependent extension products in the replication reaction is favored over formation of non-templated product.

Also disclosed is a method of performing strand displacement nucleic acid synthesis at an elevated temperature, the method comprising mixing thermolabile nucleic acid

20     polymerase having strand-displacement activity, single-stranded template nucleic acid, a set of primers, deoxyribonucleotide triphosphates and an additive, and incubating at an elevated temperature and under conditions favoring hybridization of primer to template nucleic acid and extension of primer by the addition of nucleotides sequentially to the 3' end of the primer in a template-dependent manner, wherein said polymerization results in replication

25     of said template nucleic acid.

Also disclosed is a method of amplifying nucleic acids, the method comprising incubating nucleic acids at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, a set of primers, and deoxyribonucleotide triphosphates under conditions promoting replication of nucleic acids.

30     During replication at least one of the replicated nucleic acid strands is displaced by strand displacement replication of another replicated strand. Formation of template-dependent extension products in the replication reaction is favored over formation of non-templated product.

Also disclosed is a kit for amplifying nucleic acids, the kit comprising a
thermolabile nucleic acid polymerase having strand displacement activity, an additive, and a
set of primers, wherein incubating nucleic acids comprising target sequences at an elevated
temperature in the presence of the thermolabile nucleic acid polymerase, the additive, and

5      the set of primers under conditions promoting replication of the nucleic acids results in
replicated strands and in formation of replicated strands from the target sequences in favor
of formation of replicated strands from non-target sequences.

The disclosed methods can be performed on any desired samples.  For example, the
disclosed methods can be performed using samples that contain or are suspected of

10     containing nucleic acids.  Some forms of the disclosed methods do not require knowledge of
any sequence present in a sample in order to amplify nucleic acids in the sample.
Accordingly, some forms of the disclosed methods can be used to determine if a sample
contains nucleic acids.  If amplification products are produced when the method is
performed, the sample contains nucleic acids.  The disclosed methods can be performed on

15     cells and on nucleic acid samples, including crude nucleic acid samples, partially purified
nucleic acid sample, and purified nucleic acid samples.

In some forms of the disclosed method, the primers can be hexamer primers, the
DNA polymerase can be $\phi$29 DNA polymerase, or both.  Such short primers are easier to
produce as a complete set of primers of random sequence (random primers) than longer

20     primers because there are fewer separate species of primers in a pool of shorter primers.
The above features are especially useful in whole genome strand displacement amplification
(WGSDA).

In some forms of the disclosed method, the method includes labeling of the
replicated strands (that is, the strands produced in multiple displacement amplification)

25     using terminal deoxynucleotidyl transferase.  The replicated strands can be labeled by, for
example, the addition of modified nucleotides, such as biotinylated nucleotides, fluorescent
nucleotides, 5 methyl dCTP, bromodeoxyuridine triphosphate (BrdUTP), or 5-(3-
aminoallyl)-2'-deoxyuridine 5'-triphosphates, to the 3' ends of the replicated strands.  The
replicated strands can also be labeled by incorporating modified nucleotides during

30     replication.  Probes replicated in this manner are particularly useful for hybridization,
including use in microarray formats.

Also disclosed are compositions and methods for amplification of nucleic acid
sequences of interest to produce amplification products of high quality.  It has been

discovered that amplification reactions can produce amplification products of high quality, such as low amplification bias, if performed on an amount of nucleic acid at or over a threshold amount and/or on nucleic acids at or below a threshold concentration. The threshold amount and concentration can vary depending on the nature and source of the nucleic acids to be amplified and the type of amplification reaction employed. Disclosed is a method of determining the threshold amount and/or threshold concentration of nucleic acids that can be used with nucleic acid samples of interest in amplification reactions of interest. Because amplification reactions can produce high quality amplification products, such as low bias amplification products, below the threshold amount and/or concentration of nucleic acid, such below-threshold amounts and/or concentrations can be used in amplification reactions. Accordingly, also disclosed is a method of determining amounts and/or concentrations of nucleic acids that can be used with nucleic acid samples of interest in amplification reactions of interest to produce amplification products having less than a selected amplification bias (or other measure of the quality of the amplified nucleic acids). The quality of the amplification products produced by the disclosed methods can be measured by any desired standard, and the threshold amount (or above) and/or threshold concentration (or below) to achieve a desired level of quality measured by a standard of interest can be determined by, and for used in, the disclosed methods.

It was also discovered that exposure of nucleic acids to alkaline conditions, reduction of the pH of nucleic acids exposed to alkaline conditions, and incubation of the resulting nucleic acids at or over a threshold amount and/or at or below a threshold concentration can produce amplification products with low amplification bias. Such an alkaline/neutralization procedure can improve the quality of the amplification products. The quality of the amplification products can be measured in a variety of ways, including, but not limited to, amplification bias, allele bias, locus representation, sequence representation, allele representation, locus representation bias, sequence representation bias, percent representation, percent locus representation, percent sequence representation, and other measures that indicate unbiased and/or complete amplification of the input nucleic acids.

In some forms of the disclosed method, a genomic sample is prepared by exposing the sample to alkaline conditions to denature the nucleic acids in the sample; reducing the pH of the sample to make the pH of the sample compatible with DNA replication; and incubating the sample under conditions that promote replication of the genome. In some

embodiments, the conditions of incubation can be conditions that promote replication of the genome and produce amplified genomic nucleic acids having a low amplification bias, an amplification bias at or below a desired level, or any other measure of the quality of the amplification products. Accordingly, also disclosed is a method of determining conditions

5      that can be used with nucleic acid samples of interest in amplification reactions of interest to produce amplification products having less than a selected amplification bias (or other measure of the quality of the amplified nucleic acids).

The disclosed methods can be performed on any desired samples. For example, the disclosed methods can be performed using samples that contain or are suspected of

10     containing nucleic acids. Some forms of the disclosed methods do not require knowledge of any sequence present in a sample in order to amplify nucleic acids in the sample. Accordingly, some forms of the disclosed methods can be used to determine if a sample contains nucleic acids. If amplification products are produced when the method is performed, the sample contains nucleic acids. The disclosed methods can be performed on

15     cells and on nucleic acid samples, including crude nucleic acid samples, partially purified nucleic acid sample, and purified nucleic acid samples. Exposing any cell or nucleic acid sample to alkaline conditions and then reducing the pH of the sample can produce a stabilized sample suitable for amplification or replication.

Some forms of the methods are based on strand displacement replication of the

20     nucleic acid sequences by multiple primers. Such methods, referred to as multiple displacement amplification (MDA), improves on prior methods of strand displacement replication. The disclosed method generally involves bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. Replication of the target sequence results in

25     replicated strands such that, during replication, the replicated strands are displaced from the target sequence by strand displacement replication of another replicated strand.

In some forms of the disclosed method, a genomic sample is prepared by exposing cells to alkaline conditions, thereby lysing the cells and resulting in a cell lysate; reducing the pH of the cell lysate to make the pH of the cell lysate compatible with DNA replication;

30     and incubating the cell lysate under conditions that promote replication of the genome of the cells by multiple displacement amplification. It has been discovered that alkaline lysis can cause less damage to genomic DNA and that alkaline lysis is compatible with multiple displacement amplification. The alkaline conditions can be, for example, those that cause a

substantial number of cells to lyse or those that cause a sufficient number of cells to lyse. The number of lysed cells can be considered sufficient if the genome can be sufficiently amplified in the disclosed method. The amplification is sufficient if enough amplification product is produced to permit some use of the amplification product, such as detection of

5      sequences or other analysis. The reduction in pH is generally into the neutral range of pH 9.0 to pH 6.0.

       In some embodiments, the cells are not lysed by heat and/or the nucleic acids in the cell lysate or sample are not denatured by heating. Those of skill in the art will understand that different cells under different conditions will be lysed at different temperatures and so

10     can determine temperatures and times at which the cells will not be lysed by heat. In general, the cells are not subjected to heating above a temperature and for a time that would cause substantial cell lysis in the absence of the alkaline conditions used. In some embodiments, the cells and/or cell lysate are not subjected to heating substantially above the temperature at which the cells grow. In other embodiments, the cells and/or cell lysate are

15     not subjected to heating substantially above the temperature of the amplification reaction (where the genome is replicated). The disclosed multiple displacement amplification reaction is generally conducted at a substantially constant temperature (that is, the amplification reaction is substantially isothermic), and this temperature is generally below the temperature at which the nucleic acids would be substantially or significantly denatured.

20     In some embodiments, the cell lysate or sample is not subjected to purification prior to the amplification reaction. In the context of the disclosed method, purification generally refers to the separation of nucleic acids from other material in the cell lysate or sample. It has been discovered that multiple displacement amplification can be performed on unpurified and partially purified samples. It is commonly thought that amplification

25     reactions cannot be efficiently performed using unpurified nucleic acid. In particular, PCR is very sensitive to contaminants.

       In some forms of the disclosed method, the target sample is not subjected to denaturing conditions. It was discovered that the target nucleic acids, genomic DNA, for example, need not be denatured for efficient multiple displacement amplification. It was

30     discovered that elimination of a denaturation step and denaturation conditions has additional advantages such as reducing sequence bias in the amplified products. In another embodiment, the primers can be hexamer primers. It was discovered that such short, 6 nucleotide primers can still prime multiple strand displacement replication efficiently. Such

short primers are easier to produce as a complete set of primers of random sequence (random primers) than longer primers because there are fewer separate species of primers in a pool of shorter primers. In another embodiment, the primers can each contain at least one modified nucleotide such that the primers are nuclease resistant. In another embodiment,

5        the primers can each contain at least one modified nucleotide such that the melting temperature of the primer is altered relative to a primer of the same sequence without the modified nucleotide(s). For these last two embodiments, it is preferred that the primers are modified RNA. In another embodiment, the DNA polymerase can be φ29 DNA polymerase. It was discovered that φ29 DNA polymerase produces greater amplification in

10      multiple displacement amplification. The combination of two or more of the above features also yields improved results in multiple displacement amplification. In a preferred embodiment, for example, the target sample is not subjected to denaturing conditions, the primers are hexamer primers and contain modified nucleotides such that the primers are nuclease resistant, and the DNA polymerase is φ29 DNA polymerase. The above features

15      are especially useful in whole genome strand displacement amplification (WGSDA).

In some forms of the disclosed method, the method includes labeling of the replicated strands (that is, the strands produced in multiple displacement amplification) using terminal deoxynucleotidyl transferase. The replicated strands can be labeled by, for example, the addition of modified nucleotides, such as biotinylated nucleotides, fluorescent

20      nucleotides, 5 methyl dCTP, bromodeoxyuridine triphosphate (BrdUTP), or 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphates, to the 3' ends of the replicated strands. The replicated strands can also be labeled by incorporating modified nucleotides during replication. Probes replicated in this manner are particularly useful for hybridization, including use in microarray formats.

25      In one form of the disclosed method, referred to as whole genome strand displacement amplification (WGSDA), a random set of primers is used to randomly prime a sample of genomic nucleic acid (or another sample of nucleic acid of high complexity). By choosing a sufficiently large set of primers of random or partially random sequence, the primers in the set will be collectively, and randomly, complementary to nucleic acid

30      sequences distributed throughout nucleic acid in the sample. Amplification proceeds by replication with a highly processive polymerase initiating at each primer and continuing until spontaneous termination. A key feature of this method is the displacement of intervening primers during replication by the polymerase. In this way, multiple overlapping

14

copies of the entire genome can be synthesized in a short time. The method has advantages over the polymerase chain reaction since it can be carried out under isothermal conditions. Other advantages of whole genome strand displacement amplification include a higher level of amplification than whole genome PCR (up to five times higher), amplification is less

5    sequence-dependent than PCR, and there are no re-annealing artifacts or gene shuffling artifacts as can occur with PCR (since there are no cycles of denaturation and re-annealing). In preferred embodiments of WGSDA, the target sample is not subjected to denaturing conditions, the primers are hexamer primers and contain modified nucleotides such that the primers are nuclease resistant, the DNA polymerase is $\phi$29 DNA polymerase, or any

10   combination of these features.

In another form of the method, referred to as multiple strand displacement amplification (MSDA), two sets of primers are used, a right set and a left set. Primers in the right set of primers each have a portion complementary to nucleotide sequences flanking one side of a target nucleotide sequence and primers in the left set of primers each have a

15   portion complementary to nucleotide sequences flanking the other side of the target nucleotide sequence. The primers in the right set are complementary to one strand of the nucleic acid molecule containing the target nucleotide sequence and the primers in the left set are complementary to the opposite strand. The 5' end of primers in both sets are distal to the nucleic acid sequence of interest when the primers are hybridized to the flanking

20   sequences in the nucleic acid molecule. Preferably, each member of each set has a portion complementary to a separate and non-overlapping nucleotide sequence flanking the target nucleotide sequence. Amplification proceeds by replication initiated at each primer and continuing through the target nucleic acid sequence. In another form of MSDA, referred to as linear MSDA, amplification is performed with a set of primers complementary to only

25   one strand, thus amplifying only one of the strands.

In another form of the method, referred to as gene specific strand displacement amplification (GS-MSDA), target DNA is first digested with a restriction endonuclease. The digested fragments are then ligated end-to-end to form DNA circles. These circles can be monomers or concatemers. Two sets of primers are used for amplification, a right set and a

30   left set. Primers in the right set of primers each have a portion complementary to nucleotide sequences flanking one side of a target nucleotide sequence and primers in the left set of primers each have a portion complementary to nucleotide sequences flanking the other side of the target nucleotide sequence. The primers in the right set are complementary to one

strand of the nucleic acid molecule containing the target nucleotide sequence and the primers in the left set are complementary to the opposite strand. The primers are designed to cover all or part of the sequence needed to be amplified. Preferably, each member of each set has a portion complementary to a separate and non-overlapping nucleotide sequence

5    flanking the target nucleotide sequence. Amplification proceeds by replication initiated at each primer and continuing through the target nucleic acid sequence. In one form of GS-MSDA, referred to as linear GS-MSDA, amplification is performed with a set of primers complementary to only one strand, thus amplifying only one of the strands. In another form of GS-MSDA, cDNA sequences can be circularized to form single stranded DNA circles.

10   Amplification is then performed with a set of primers complementary to the single-stranded circular cDNA.

A key feature of this method is the displacement of intervening primers during replication. Once the nucleic acid strands elongated from the right set of primers reaches the region of the nucleic acid molecule to which the left set of primers hybridizes, and vice

15   versa, another round of priming and replication will take place. This allows multiple copies of a nested set of the target nucleic acid sequence to be synthesized in a short period of time. By using a sufficient number of primers in the right and left sets, only a few rounds of replication are required to produce hundreds of thousands of copies of the nucleic acid sequence of interest. The disclosed method has advantages over the polymerase chain

20   reaction since it can be carried out under isothermal conditions. No thermal cycling is needed because the polymerase at the head of an elongating strand (or a compatible strand-displacement protein) will displace, and thereby make available for hybridization, the strand ahead of it. Other advantages of multiple strand displacement amplification include the ability to amplify very long nucleic acid segments (on the order of 50 kilobases) and rapid

25   amplification of shorter segments (10 kilobases or less). In multiple strand displacement amplification, single priming events at unintended sites will not lead to artifactual amplification at these sites (since amplification at the intended site will quickly outstrip the single strand replication at the unintended site). In preferred embodiments of MSDA, the target sample is not subjected to denaturing conditions, the primers are hexamer primers and

30   contain modified nucleotides such that the primers are nuclease resistant, the DNA polymerase is φ29 DNA polymerase, or any combination of these features.

In preferred embodiments of WGSDA, the target sample is not subjected to denaturing conditions, the primers are hexamer primers and contain modified nucleotides

such that the primers are nuclease resistant, the DNA polymerase is φ29 DNA polymerase, or any combination of these features.

Following amplification, the amplified sequences can be used for any purpose, such as uses known and established for PCR amplified sequences.  For example, amplified

5      sequences can be detected using any of the conventional detection systems for nucleic acids such as detection of fluorescent labels, enzyme-linked detection systems, antibody-mediated label detection, and detection of radioactive labels.  A preferred form of labeling involves labeling of the replicated strands (that is, the strands produced in multiple displacement amplification) using terminal deoxynucleotidyl transferase.  The replicated strands can be

10     labeled by, for example, the addition of modified nucleotides, such as biotinylated nucleotides, fluorescent nucleotides, 5 methyl dCTP, BrdUTP, or 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphates, to the 3' ends of the replicated strands.

In the disclosed method amplification takes place not in cycles, but in a continuous, isothermal replication.  This makes amplification less complicated and much more

15     consistent in output.  Strand displacement allows rapid generation of multiple copies of a nucleic acid sequence or sample in a single, continuous, isothermal reaction.  DNA that has been produced using the disclosed method can then be used for any purpose or in any other method desired.  For example, PCR can be used to further amplify any specific DNA sequence that has been previously amplified by the whole genome strand displacement

20     method.

Genetic analysis must frequently be carried out with DNA derived from biological samples, such as blood, tissue culture cells, buccal swabs, mouthwash, stool, tissues slices, biopsy aspiration, and archeological samples such as bone or mummified tissue.  In some cases, the samples are too small to extract a sufficient amount of pure DNA and it is

25     necessary to carry out DNA-based assays directly from the unprocessed sample. Furthermore, it is time consuming to isolate pure DNA, and so the disclosed method, which can amplify the genome directly from biological samples, represents a substantial improvement.

The disclosed method has several distinct advantages over current methodologies.

30     The genome can be amplified directly from whole blood or cultured cells with simple cell lysis techniques such as KOH treatment.  PCR and other DNA amplification methods are severely inhibited by cellular contents and so purification of DNA is needed prior to amplification and assay.  For example, heme present in lysed blood cells inhibits PCR.  In

contrast, the disclosed form of whole genome amplification can be carried out on crude lysates with no need to physically separate DNA by miniprep extraction and precipitation procedures, or with column or spin cartridge methods.

5      Bacteria, fungi, and viruses may all be involved in nosocomial infections. Identification of nosocomial pathogens at the sub-species level requires sophisticated discriminatory techniques. Such techniques utilize traditional as well as molecular methods for typing. Some traditional techniques are antimicrobial susceptibility testing, determination of the ability to utilize biochemical substrates, and serotyping. A major limitation of these techniques is that they take several days to complete, since they require

10    pure bacterial cultures. Because such techniques are long, and the bacteria may even be non-viable in the clinical samples, there is a need to have a quick and reliable method for bacterial species identification.

Some of the DNA-based molecular methods for the identification of bacterial species are macrorestriction analysis (MRA) followed by pulsed-field gel electrophoresis

15    (PFGE), amplified fragment length polymorphism (AFLP) analysis, and arbitrarily primed PCR (AP-PCR) (Tenover et al., J. Clin. Microbiol. 32:407-415 (1994), and Pruckler et al., J. Clin. Microbiol. 33:2872-2875 (1995)). These molecular techniques are labor-intensive and difficult to standardize among different laboratories.

The disclosed method provides a useful alternative method for the identification of

20    bacterial strains by amplification of microbial DNA for analysis. Unlike PCR (Lantz et al., Biotechnol. Annu. Rev. 5:87-130 (2000)), the disclosed method is rapid, non-biased, reproducible, and capable of amplifying large DNA segments from bacterial, viral or fungal genomes.

The disclosed method can be used, for example, to obtain enough DNA from

25    unculturable organisms for sequencing or other studies. Most microorganisms cannot be propagated outside their native environment, and therefore their nucleic acids cannot be sequenced. Many unculturable organisms live under extreme conditions, which makes their genetic complement of interest to investigators. Other microorganisms live in communities that play a vital role in certain ecosystems. Individual organisms or entire

30    communities of organisms can be amplified and sequenced, individually or together.

Recombinant proteins may be purified from a large biomass grown up from bacterial or yeast strains harboring desired expression vectors. A high degree of purity may be desired for the isolated recombinant protein, requiring a sensitive procedure for the

18

detection of trace levels of protein or DNA contaminants. The disclosed method is a DNA amplification reaction that is highly robust even in the presence of low levels of DNA template, and can be used to monitor preparations of recombinant protein for trace amounts of contaminating bacterial or yeast genomic DNA.

Amplification of forensic material for RFLP-based testing is one useful application for the disclosed method.

Also disclosed is a method for amplifying and repairing damaged DNA. This method is useful, for example, for amplifying degraded genomic DNA. The method involves substantially denaturing a damaged DNA sample (generally via exposure to heat and alkaline conditions), removal or reduction of the denaturing conditions (such as by reduction of the pH and temperature of the denatured DNA sample), and replicating the DNA. The damaged DNA is repaired during replication by increasing the average length of the damaged DNA. For example, the average length of DNA fragments can be increase from, for example, 2 kb in the damaged DNA sample to, for example, 10 kb or greater for the replicated DNA. This repair method can result in an overall improvement in amplification of damaged DNA by increasing the average length of the product, increasing the quality of the amplification products by 3-fold (by, for example, increasing the marker representation in the sample), and improving the genotyping of amplified products by lowering the frequency of allelic dropout; all compared to the results when amplifying damaged DNA by other methods. The removal of denaturing conditions can allow denatured strands of damaged DNA to hybridize to other denatured damaged DNA. The replication can be multiple displacement amplification. Substantial denaturation and transient denaturation of the DNA samples generally is carried out such that the DNA is not further damaged. This method can generally be combined or used with any of the disclosed amplification methods.

It has been discovered that it is unnecessary to have prior knowledge of whether or not a sample contains amplifiable nucleic acids. Some forms of the disclosed methods can be employed to test whether or not a sample suspected of containing nucleic acids actually does contain nucleic acids. Production of amplified DNA from such samples using the disclosed method is evidence that the sample contained nucleic acids. More generally, practice of the disclosed methods does not require any knowledge of any nucleic acid sequence in a sample. Thus, the disclosed methods can be used to amplify nucleic acids from any source, regardless of a lack of specific sequence information. This is in contrast to

19

other amplification methods, such as PCR, where it is necessary to have prior information of at least a portion of the nucleic acid sequences believed to be present in the sample in order to perform the amplification. In this instance, the PCR amplification reaction will fail if the nucleic acids present in the sample are different from the expected sample nucleic

5      acids. If a sample contains a mixture of nucleic acids, then nucleic acids of the appropriate type alone will be amplified in a PCR reaction, but not the other types of nucleic acids. In contrast, the disclosed methods provide for amplification of most or all of the nucleic acids present in the sample. The disclosed methods are equally adaptable to using samples that conventionally are not expected or believed to contain nucleic acids. For instance, serum or

10     plasma from humans or other higher animals were believed to not contain free host nucleic acids. However, it was discovered that the disclosed methods could amplify nucleic acids present in such samples.

It is an object of the disclosed invention to provide a method and kits for improving specific input template-dependent synthesis over artifact DNA synthesis.

15     It is another object of the disclosed invention to provide a method and kits that produce amplification products with reduced or undetectable levels of artifactual nucleic acids.

It is an object of the disclosed invention to provide a method of amplifying a target nucleic acid sequence in a continuous, isothermal reaction with reduced or undetectable

20     levels of artifactual nucleic acids.

It is another object of the disclosed invention to provide a method of amplifying an entire genome or other highly complex nucleic acid sample in a continuous, isothermal reaction with reduced or undetectable levels of artifactual nucleic acids.

It is another object of the disclosed invention to provide a method of amplifying a

25     target nucleic acid sequence in a continuous, isothermal reaction.

It is another object of the disclosed invention to provide a method of amplifying an entire genome or other highly complex nucleic acid sample in a continuous, isothermal reaction.

It is another object of the disclosed invention to provide a method of amplifying a

30     target nucleic acid sequence where multiple copies of the target nucleic acid sequence are produced in a single amplification cycle.

It is another object of the disclosed invention to provide a method of amplifying a concatenated DNA in a continuous, isothermal reaction.

20

It is another object of the disclosed invention to provide a kit for amplifying a target nucleic acid sequence in a continuous, isothermal reaction.

It is another object of the disclosed invention to provide a kit for amplifying an entire genome or other highly complex nucleic acid sample in a continuous, isothermal

5        reaction.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph of the locus representation (in percent) for 47 genetic loci (2 loci per chromosome, and one locus from the Y chromosome) in human genomic DNA amplified using a single six nucleotide primer of specific nucleotide sequence in an

10        embodiment of the disclosed method.

Figure 2 is a graph of the locus representation (in percent) for 47 genetic loci (2 loci per chromosome, and one locus from the Y chromosome) in human genomic DNA amplified using two different six nucleotide primers of specific nucleotide sequence in an embodiment of the disclosed method.

15        Figure 3 is a graph of the locus representation (in percent) for 47 genetic loci (2 loci per chromosome, and one locus from the Y chromosome) in human genomic DNA amplified using three different six nucleotide primers of specific nucleotide sequence in an embodiment of the disclosed method.

Figure 4 is a graph of the locus representation (in percent) for 47 genetic loci (2 loci

20        per chromosome, and one locus from the Y chromosome) in human genomic DNA amplified using four different six nucleotide primers of specific nucleotide sequence in an embodiment of the disclosed method.

Figure 5 is a graph of the locus representation (in percent) for 47 genetic loci (2 loci per chromosome, and one locus from the Y chromosome) in human genomic DNA

25        amplified using five different six nucleotide primers of specific nucleotide sequence in an embodiment of the disclosed method.

Figure 6 is a graph of the locus representation (in percent) for 47 genetic loci (2 loci per chromosome, and one locus from the Y chromosome) in human genomic DNA amplified using five different six nucleotide primers of specific nucleotide sequence in an

30        embodiment of the disclosed method.

Figure 7 is a graph of the locus representation (in percent) for 47 genetic loci (2 loci per chromosome, and one locus from the Y chromosome) in human genomic DNA

amplified using five different six nucleotide primers of specific nucleotide sequence in an embodiment of the disclosed method.

Figure 8 is a graph of the locus representation (in percent) for 47 genetic loci (2 loci per chromosome, and one locus from the Y chromosome) in human genomic DNA

5      amplified using five different six nucleotide primers of specific nucleotide sequence in an embodiment of the disclosed method.

Figure 9 is a graph of the locus representation (in percent) for 10 genetic loci in human genomic DNA amplified using nine different six nucleotide primers of specific nucleotide sequence in an embodiment of the disclosed method.

10      Figure 10 is a graph of the locus representation (in percent) for 47 genetic loci (2 loci per chromosome, and one locus from the Y chromosome) in human genomic DNA amplified using twelve different six nucleotide primers of specific nucleotide sequence in an embodiment of the disclosed method.

Figure 11 is a graph of DNA synthesis by MDA reaction carried out for 16 hrs.

15      using varying amounts (0, 0.3 ng, 3 ng and 30 ng) of intact genomic DNA (gDNA) or genomic DNA that was degraded by heating at 85°C for 10 minutes (degraded gDNA) as the input template. The MDA reaction was either carried out at 30 °C in the absence of 0.3 M Trehalose or at 40°C in the presence of 0.3 M Trehalose.

Figure 12 is a graph of DNA synthesis by MDA reaction carried out at 40°C in the

20      presence of various sugars for 16 hrs. using varying amounts (0 to 30 ng) of intact genomic DNA as the input template. The MDA reaction was either carried out with no additive or in the presence of 0.3 M Trehalose or 0.4 M Sucrose or 0.4 M Glucose.

Figure 13 is a graph of DNA synthesis (in μg) versus time (in hours) using different amounts of nucleic acid for amplification in the disclosed method.

25      Figure 14 is a graph of the effect of incubation time at 95°C on template DNA length.

Figure 15 is a graph of the effect of template incubation at 95°C on the rate and yield of MDA.

Figure 16 is a graph of the effect of template incubation at 95°C on the average size

30      of DNA product strands.

Figure 17 is a graph showing a comparison of the effect of template incubation at 95°C versus no incubation at 95°C on locus representation in DNA amplified by MDA.

Figures 18A, 18B, and 18C are graphs showing the effect of amplification on gene representation bias for three different amplification procedures, MDA, DOP-PCR, and PEP.

Figure 19 is a graph showing amplification of c-jun sequences using nested primers.

Figure 20 is a graph the relative representation of eight loci for DNA from five different amplification reactions. The Y-axis is the locus representation, expressed as a percent, relative to input genomic DNA, which is calculated as the yield of quantitative PCR product from 1 µg of amplified DNA divided by the yield from 1 µg of genomic DNA control.

Figure 21 is a graph showing a comparison of the percent representation for 8 loci for DNA amplified in a reaction containing 100% dTTP and DNA amplified in a reaction containing 30% dTTP / 70% AAdUTP.

Figure 22 is a graph showing the amplification of c-jun sequences using circularized genomic template. The Y-axis is the locus representation, expressed as a percent, relative to input genomic DNA, which is calculated as the yield of quantitative PCR product from 1 µg of amplified DNA divided by the yield from 1 µg of genomic DNA control.

Figure 23 is a graph showing a comparison of the percent representation for 8 loci in DNA amplified using c-jun specific primers and circularized DNA target.

Figure 24 is a graph of percent locus representation of different DNA samples exposed to different treatments (control or repair treatments).

Figure 25 is a graph of percent locus representation of 40 samples with or without repair treatment.

Figure 26 is a graph comparing the amount of alleles at each of five single nucleotide polymorphisms found in a genomic nucleic acid sample amplified using a form of the disclosed method (with alkaline treatment; bottom panel) or the same method without alkaline treatment (top panel). The amplification bias of the alleles is lower when an alkaline treatment is used. 168 Coriell gDNA in 100 µL reactions. Genotyping was performed by TaqMan assay.

Figure 27 is a graph comparing the amount of alleles at each of five single nucleotide polymorphisms found in a genomic nucleic acid sample amplified using a form of the disclosed method (with alkaline treatment; bottom panel) or the same method without alkaline treatment (top panel). The amplification bias of the alleles is lower when an alkaline treatment is used. 168 Coriell gDNA in 100 µL reactions. Genotyping was performed by TaqMan assay.

## DETAILED DESCRIPTION OF THE INVENTION

The disclosed method makes use of certain materials and procedures which allow amplification of target nucleic acid sequences and whole genomes or other highly complex nucleic acid samples. These materials and procedures are described in detail below.

**Materials**

### A. Target Sequence

The target sequence, which is the object of amplification, can be any nucleic acid. The target sequence can include multiple nucleic acid molecules, such as in the case of whole genome amplification, multiple sites in a nucleic acid molecule, or a single region of a nucleic acid molecule. For multiple strand displacement amplification, generally the target sequence is a single region in a nucleic acid molecule or nucleic acid sample. For whole genome amplification, the target sequence is the entire genome or nucleic acid sample. A target sequence can be in any nucleic acid sample of interest. The source, identity, and preparation of many such nucleic acid samples are known. It is preferred that nucleic acid samples known or identified for use in amplification or detection methods be used for the method described herein. The nucleic acid sample can be, for example, a nucleic acid sample from one or more cells, tissue, or bodily fluids such as blood, urine, semen, lymphatic fluid, cerebrospinal fluid, or amniotic fluid, or other biological samples, such as tissue culture cells, buccal swabs, mouthwash, stool, tissues slices, biopsy aspiration, and archeological samples such as bone or mummified tissue. Target samples can be derived from any source including, but not limited to, eukaryotes, plants, animals, vertebrates, fish, mammals, humans, non-humans, bacteria, microbes, viruses, biological sources, serum, plasma, blood, urine, semen, lymphatic fluid, cerebrospinal fluid, amniotic fluid, biopsies, needle aspiration biopsies, cancers, tumors, tissues, cells, cell lysates, crude cell lysates, tissue lysates, tissue culture cells, buccal swabs, mouthwash, stool, mummified tissue, forensic sources, autopsies, archeological sources, infections, nosocomial infections, production sources, drug preparations, biological molecule productions, protein preparations, lipid preparations, carbohydrate preparations, inanimate objects, air, soil, sap, metal, fossils, excavated materials, and/or other terrestrial or extra-terrestrial materials and sources. The sample may also contain mixtures of material from one or more different sources. For example, nucleic acids of an infecting bacterium or virus can be amplified along with human nucleic acids when nucleic acids from such infected cells or tissues are amplified using the disclosed methods. Types of useful target samples include eukaryotic

samples, plant samples, animal samples, vertebrate samples, fish samples, mammalian samples, human samples, non-human samples, bacterial samples, microbial samples, viral samples, biological samples, serum samples, plasma samples, blood samples, urine samples, semen samples, lymphatic fluid samples, cerebrospinal fluid samples, amniotic fluid

5          samples, biopsy samples, needle aspiration biopsy samples, cancer samples, tumor samples, tissue samples, cell samples, cell lysate samples, crude cell lysate samples, tissue lysate samples, tissue culture cell samples, buccal swab samples, mouthwash samples, stool samples, mummified tissue samples, forensic samples, autopsy samples, archeological samples, infection samples, nosocomial infection samples, production samples, drug

10         preparation samples, biological molecule production samples, protein preparation samples, lipid preparation samples, carbohydrate preparation samples, inanimate object samples, air samples, soil samples, sap samples, metal samples, fossil samples, excavated material samples, and/or other terrestrial or extra-terrestrial samples.

For multiple strand displacement amplification, preferred target sequences are those

15         which are difficult to amplify using PCR due to, for example, length or composition. For whole genome amplification, preferred target sequences are nucleic acid samples from a single cell. For multiple strand displacement amplification of concatenated DNA the target is the concatenated DNA. The target sequence can be either one or both strands of cDNA. The target sequences for use in the disclosed method are preferably part of nucleic acid

20         molecules or samples that are complex and non-repetitive (with the exception of the linkers in linker-concatenated DNA and sections of repetitive DNA in genomic DNA).

Target nucleic acids can include damaged DNA and damaged DNA samples. For example, preparation of genomic DNA samples can result in damage to the genomic DNA (for example, degradation and fragmentation). This can make amplification of the genome

25         or sequences in it both more difficult and provide less reliable results (by, for example, resulting in amplification of many partial and fragmented genomic sequences. Damaged DNA and damaged DNA samples are thus useful for the disclosed method of amplifying damaged DNA. Any degraded, fragmented or otherwise damaged DNA or sample containing such DNA can be used in the disclosed method.

30         **1. Target Sequences for Multiple Strand Displacement Amplification**

Although multiple sites in a nucleic acid sample can be amplified simultaneously in the same MSDA reaction, for simplicity, the following discussion will refer to the features of a single nucleic acid sequence of interest which is to be amplified. This sequence is

referred to below as a target sequence. It is preferred that a target sequence for MSDA include two types of target regions, an amplification target and a hybridization target. The hybridization target includes the sequences in the target sequence that are complementary to the primers in a set of primers. The amplification target is the portion of the target sequence which is to be amplified. For this purpose, the amplification target is preferably downstream of, or flanked by the hybridization target(s). There are no specific sequence or structural requirements for choosing a target sequence. The hybridization target and the amplification target within the target sequence are defined in terms of the relationship of the target sequence to the primers in a set of primers. The primers are designed to match the chosen target sequence. Although preferred, it is not required that sequences to be amplified and the sites of hybridization of the primers be separate since sequences in and around the sites where the primers hybridize will be amplified.

In multiple strand displacement amplification of circularized DNA, the circular DNA fragments are the amplification targets. The hybridization targets include the sequences that are complementary to the primers used for amplification. One form of circular DNA for amplification is circularized cDNA.

In multiple strand displacement amplification of linker-concatenated DNA, the DNA fragments joined by the linkers are the amplification targets and the linkers are the hybridization target. The hybridization targets (that is, the linkers) include the sequences that are complementary to the primers used for amplification. One form of concatenated DNA for amplification is concatenated cDNA.

**B. Additives**

Additives for use in the disclosed amplification method are any compound, composition, or combination that can allow a thermolabile nucleic acid polymerase to perform template-dependent polymerization at an elevated temperature. Additives generally have a thermostabilizing effect on the nucleic acid polymerase. Additives allow a thermolabile nucleic acid polymerase to be used at temperature above the normal active range of the polymerase. Useful additives include sugars, chaperones, proteins, saccharides, amino acids, polyalcohols and their derivatives, and other osmolytes. Useful sugars include trehalose, glucose and sucrose. Useful saccharides include oligosaccharides and monosaccharides such as trehalose, maltose, glucose, sucrose, lactose, xylobiose, agarobiose, cellobiose, levanbiose, quitobiose, 2-$\beta$-glucuronosylglucuronic acid, allose, altrose, galactose, gulose, idose, mannose, talose, sorbitol, levulose, xylitol, arabitol, and

26

polyalcohols such as glycerol, ethylene glycol, polyethylene glycol. Useful amino acids and derivatives thereof include $N^e$-acetyl-$\beta$-lysine, alanine, $\gamma$-aminobutyric acid, betaine, $N^\alpha$-carbamoyl-L-glutamine 1-amide, choline, dimethylthetine, ecotine (1,4,5,6-tetrahydro-2-methyl-4-pirymidine carboxilic acid), glutamate, $\beta$-glutammine, glycine, octopine, proline,

5      sarcosine, taurine and trymethylamine N-oxide (TMAO). Useful chaperone proteins include chaperone proteins of Thermophilic bacteria and heat shock proteins such as HSP 90, HSP 70 and HSP 60. Other useful additives include sorbitol, mannosylglycerate, diglycerol phosphate, and cyclic-2,3-diphosphoglycerate. Combinations of compounds and compositions can be used as additives.

10     As used herein, an elevated temperature is a temperature at or above which a given nucleic acid polymerase is notably inactivated in the absence of an additive, dNTPs, and template nucleic acid. Thus, what constitutes an elevated temperature depends on the particular nucleic acid polymerase. As used herein, notable inactivation refers to a reduction in activity of 40% or more. Substantial inactivation refers to a reduction in

15     activity of 60% or more. Significant inactivation refers to a reduction in activity of 80% or more.

As used herein, a thermolabile nucleic acid polymerase is a nucleic acid polymerase that is notably inactivated at the temperature at which an amplification reaction is carried out in the absence of an additive, dNTPs, and template nucleic acid. Thus, whether a

20     nucleic acid polymerase is thermolabile depends on the temperature at which an amplification reaction is carried out. Note that as used herein, thermolability does not require denaturation or irreversible inactivation of a polymerase. All that is required is that the polymerase be notably incapable of performing template-dependent polymerization at the temperature at which an amplification reaction is carried out in the absence of an

25     additive.

**C. Samples**

Nucleic acid molecules, which are the object of amplification, can be any nucleic acid from any source. In general, the disclosed method is performed using a sample that contains (or is suspected of containing) nucleic acid molecules to be amplified. Samples

30     containing, or suspected of containing, nucleic acid molecules can also be referred to as nucleic acid samples. Samples, such as nucleic acid samples can comprise target sequences. Cell and tissue samples are a form of nucleic acid sample. Samples for use in the disclosed methods can also be samples that are to be tested for the presence of nucleic acids (that is,

samples that may or may not contain nucleic acids). For whole genome amplification, the sample can be all or a substantial portion of an entire genome. As used herein, a substantial portion of a genome refers to the presence of 90% or more of the sequences present in the entire genome. A sample, such as a nucleic acid sample or genomic nucleic acid sample,

5      including or comprising a substantial portion of a genome refers to a sample including 90% or more of the sequences present in the entire genome. A genomic nucleic acid sample refers to any sample derived from genomic nucleic acids and including or comprising a notable portion of the entire genome. As used herein, a notable portion of a genome refers to the presence of 20% or more of the sequences present in the entire genome. A sample,

10     such as a nucleic acid sample or genomic nucleic acid sample, including or comprising a notable portion of a genome refers to a sample including 20% or more of the sequences present in the entire genome. As used herein, a significant portion of a genome refers to the presence of 50% or more of the sequences present in the entire genome. A sample, such as a nucleic acid sample or genomic nucleic acid sample, including or comprising a significant

15     portion of a genome refers to a sample including 50% or more of the sequences present in the entire genome. A genomic nucleic acid sample is a form of nucleic acid sample and a form of sample. Reference herein to a sample encompasses nucleic acid samples and genomic samples unless the context clearly indicates otherwise. Reference herein to a nucleic acid sample encompasses genomic nucleic acid samples unless the context clearly

20     indicates otherwise.

        A sample can comprise a genome, and the genome can comprise any fraction of the nucleic acids in the sample. The genome can comprise, for example, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%,

25     at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the nucleic acids in the sample.

        The nucleic acids in a sample need not be pure to be amplified in the disclosed methods. Some forms of the disclosed methods are useful for amplifying impure nucleic acid samples, such as crude cell lysates. The nucleic acids in a sample or in a stabilized or

30     neutralized sample can be, for example, less than 0.01% pure, less than 0.5% pure, less than 0.1% pure, less than 0.2% pure, less than 0.4% pure, less than 0.6% pure, less than 0.8% pure, less than 1% pure, less than 2% pure, less than 3% pure, less than 4% pure, less than 5% pure, less than 6% pure, less than 8% pure, less than 10% pure, less than 15% pure, less

than 20% pure, less than 25% pure, less than 30% pure, less than 40% pure, or less than 50% pure by weight excluding water.

A nucleic acid sample can be any nucleic acid sample of interest. The source, identity, and preparation of many such nucleic acid samples are known. It is preferred that nucleic acid samples known or identified for use in amplification or detection methods be used for the method described herein. The nucleic acid sample can be, for example, a nucleic acid sample comprising or derived from one or more eukaryotes, plants, animals, vertebrates, fish, mammals, humans, non-humans, bacteria, microbes, viruses, biological sources, serum, plasma, blood, urine, semen, lymphatic fluid, cerebrospinal fluid, amniotic fluid, biopsies, needle aspiration biopsies, cancers, tumors, tissues, cells, cell lysates, crude cell lysates, tissue lysates, tissue culture cells, buccal swabs, mouthwash, stool, mummified tissue, forensic sources, autopsies, archeological sources, infections, nosocomial infections, production sources, drug preparations, biological molecule productions, protein preparations, lipid preparations, carbohydrate preparations, inanimate objects, air, soil, sap, metal, fossils, excavated materials, and/or other terrestrial or extra-terrestrial materials and sources. Types of useful nucleic acid samples include eukaryotic samples, plant samples, animal samples, vertebrate samples, fish samples, mammalian samples, human samples, non-human samples, bacterial samples, microbial samples, viral samples, biological samples, serum samples, plasma samples, blood samples, urine samples, semen samples, lymphatic fluid samples, cerebrospinal fluid samples, amniotic fluid samples, biopsy samples, needle aspiration biopsy samples, cancer samples, tumor samples, tissue samples, cell samples, cell lysate samples, crude cell lysate samples, tissue lysate samples, tissue culture cell samples, buccal swab samples, mouthwash samples, stool samples, mummified tissue samples, forensic samples, autopsy samples, archeological samples, infection samples, nosocomial infection samples, production samples, drug preparation samples, biological molecule production samples, protein preparation samples, lipid preparation samples, carbohydrate preparation samples, inanimate object samples, air samples, soil samples, sap samples, metal samples, fossil samples, excavated material samples, and/or other terrestrial or extra-terrestrial samples.

It has been discovered that it is unnecessary to have prior knowledge of whether or not a sample contains amplifiable nucleic acids. Some forms of the disclosed methods can be employed to test whether or not a sample suspected of containing nucleic acids actually does contain nucleic acids. Production of amplified DNA from such samples using the

disclosed method is evidence that the sample contained nucleic acids. More generally, practice of the disclosed methods does not require any knowledge of any nucleic acid sequence in a sample. Thus, the disclosed methods can be used to amplify nucleic acids from any source, regardless of a lack of specific sequence information. This is in contrast to other amplification methods, such as PCR, where it is necessary to have prior information of at least a portion of the nucleic acid sequences believed to be present in the sample in order to perform the amplification. In this instance, the PCR amplification reaction will fail if the nucleic acids present in the sample are different from the expected sample nucleic acids. If a sample contains a mixture of nucleic acids, then nucleic acids of the appropriate type alone will be amplified in a PCR reaction, but not the other types of nucleic acids. In contrast, the disclosed methods provide for amplification of most or all of the nucleic acids present in the sample. The disclosed methods are equally adaptable to using samples that conventionally are not expected or believed to contain nucleic acids. For instance, serum or plasma from humans or other higher animals were believed to not contain free host nucleic acids. However, it was discovered that the disclosed methods could amplify nucleic acids present in such samples.

For whole genome amplification, preferred nucleic acid samples are nucleic acid samples from a single cell. The nucleic acid samples for use in some forms of the disclosed method are preferably nucleic acid molecules and samples that are complex and non-repetitive. Where the nucleic acid sample is a genomic nucleic acid sample, the genome can be the genome from any organism of interest. For example, the genome can be a viral genome, a bacterial genome, a eubacterial genome, an archae bacterial genome, a fungal genome, a microbial genome, a eukaryotic genome, a plant genome, an animal genome, a vertebrate genome, an invertebrate genome, an insect genome, a mammalian genome, or a human genome. The target genome is preferably pure or substantially pure, but this is not required. For example, an genomic sample from an animal source may include nucleic acid from contaminating or infecting organisms.

The nucleic acid sample can be, or can be derived from, for example, one or more whole genomes from the same or different organisms, tissues, cells or a combination; one or more partial genomes from the same or different organisms, tissues, cells or a combination; one or more whole chromosomes from the same or different organisms, tissues, cells or a combination; one or more partial chromosomes from the same or different organisms, tissues, cells or a combination; one or more chromosome fragments from the same or

30

different organisms, tissues, cells or a combination; one or more artificial chromosomes; one or more yeast artificial chromosomes; one or more bacterial artificial chromosomes; one or more cosmids; or any combination of these.

Where the nucleic acid sample is a nucleic acid sample of high complexity, the nucleic acid molecules in the sample can be from any source or combination of sources that result in a highly complex sample. By high complexity or high sequence complexity is meant that the nucleic acid sample has a large number of unique (that is, non-repeated) sequences. The total number of nucleotides in the unique sequences is the sequence complexity of the nucleic acid sample. For example, the human genome has approximately $3 \times 10^9$ unique sequences and so has a sequence complexity of approximately $3 \times 10^9$ nucleotides. A nucleic acid sample of high sequence complexity has a sequence complexity of at least $1 \times 10^6$ nucleotides. Thus, a nucleic acid sample of high sequence complexity can have, for example, a sequence complexity of at least $1 \times 10^6$ nucleotides, a sequence complexity of at least $1 \times 10^7$ nucleotides, a sequence complexity of at least $1 \times 10^8$ nucleotides, or a sequence complexity of at least $1 \times 10^9$ nucleotides.

The nucleic acid sample can also be a nucleic acid sample of significant complexity. By significant complexity or significant sequence complexity is meant that the nucleic acid sample has a significant number of unique (that is, non-repeated) sequences. A nucleic acid sample of significant sequence complexity has a sequence complexity of at least $1 \times 10^5$ nucleotides. Thus, a nucleic acid sample of significant sequence complexity can have, for example, a sequence complexity of at least $1 \times 10^5$ nucleotides, a sequence complexity of at least $1 \times 10^6$ nucleotides, a sequence complexity of at least $1 \times 10^7$ nucleotides, a sequence complexity of at least $1 \times 10^8$ nucleotides, or a sequence complexity of at least $1 \times 10^9$ nucleotides. The nucleic acid sample can also be a nucleic acid sample of notable complexity. By notable complexity or notable sequence complexity is meant that the nucleic acid sample has a notable number of unique (that is, non-repeated) sequences. A nucleic acid sample of notable sequence complexity has a sequence complexity of at least $1 \times 10^4$ nucleotides. Thus, a nucleic acid sample of significant sequence complexity can have, for example, a sequence complexity of at least $1 \times 10^4$ nucleotides, a sequence complexity of at least $1 \times 10^5$ nucleotides, a sequence complexity of at least $1 \times 10^6$ nucleotides, a sequence complexity of at least $1 \times 10^7$ nucleotides, a sequence complexity of at least $1 \times 10^8$ nucleotides, or a sequence complexity of at least $1 \times 10^9$ nucleotides.

Nucleic acid samples and genomic nucleic acid samples can have, for example, a sequence complexity of at least $1 \times 10^3$ nucleotides, a sequence complexity of at least $1 \times 10^4$ nucleotides, a sequence complexity of at least $1 \times 10^5$ nucleotides, a sequence complexity of at least $1 \times 10^6$ nucleotides, a sequence complexity of at least $1 \times 10^7$

5    nucleotides, a sequence complexity of at least $1 \times 10^8$ nucleotides, or a sequence complexity of at least $1 \times 10^9$ nucleotides.

Samples can be used and manipulated in the disclosed methods. For example, a sample can be exposed to alkaline conditions or brought into contact or mixed with a lysis solution or denaturing solution. As used herein, the term sample refers both to source

10   samples, samples used in the disclosed methods in whole, and to portions of source samples used in the disclosed methods. Thus, for example, a portion of a source sample that is exposed to alkaline conditions is considered to be a sample itself. All or a portion of a sample can be exposed to alkaline conditions or brought into contact or mixed with a lysis solution or denaturing solution. Similarly, the pH of all or a portion of a sample exposed to

15   alkaline conditions or brought into contact or mixed with a lysis solution or denaturing solution can be reduced, or all or a portion of a sample exposed to alkaline conditions or brought into contact with a lysis solution or denaturing solution can be brought into contact or mixed with a stabilization solution. All or a portion of the resulting stabilized or neutralized sample can be incubated under conditions that promote replication of nucleic

20   acids. An amplification mixture can comprise all or a portion of a stabilized or neutralized sample. An amplification mixture is the reaction solution where nucleic acids are amplified.

**D.  Primers**

Primers for use in the disclosed amplification method are oligonucleotides having sequence complementary to the target sequence. This sequence is referred to as the

25   complementary portion of the primer. The complementary portion of a primer can be any length that supports specific and stable hybridization between the primer and the target sequence under the reaction conditions. Generally, for reactions at 37°C, this can be 10 to 35 nucleotides long or 16 to 24 nucleotides long. Generally, for reactions at 30°C, this can be, for example, 5 to 20 nucleotides long or 6 to 8 nucleotides long. For whole genome

30   amplification, the primers can be, for example, from 2 to 60 nucleotides long or 5 to 60 nucleotides long, and in particular, can be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and/or 20 nucleotides long. The primers also can be, for example, at least 2 nucleotides long, at least 3 nucleotides long, at least 4 nucleotides long, at least 5

nucleotides long, at least 6 nucleotides long, at least 7 nucleotides long, and/or at least 8 nucleotides long. The primers used in an amplification reaction need not be all of the same length, although this is preferred.

For some forms of the disclosed method, such as those using primers or random or degenerate sequence (that is, use of a collection of primers having a variety of sequences), primer hybridization need not be specific. In such cases the primers need only be effective in priming synthesis. For example, in whole genome amplification specificity of priming is not essential since the goal generally is to amplify all sequences equally. Sets of random or degenerate primers can be composed of primers 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and/or 20 nucleotides long or more. Primers six nucleotides long are referred to as hexamer primers. Preferred primers for whole genome amplification are random hexamer primers, for example, random hexamer primers where every possible six nucleotide sequence is represented in the set of primers. Similarly, sets of random primers of other particular lengths, or of a mixture of lengths preferably contain every possible sequence the length of the primer, or, in particular, the length of the complementary portion of the primer. Use of random primers is described in U.S. Patent No. 5,043,272 and U.S. Patent No. 6,214,587.

The primers can have, for example, a length of 3 nucleotides, 4 nucleotides, 5 nucleotides, 6 nucleotides, 7 nucleotides, 8 nucleotides, 9 nucleotides, 10 nucleotides, 11 nucleotides, 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides. The primers can have, for example, a length of less than 4 nucleotides, less than 5 nucleotides, less than 6 nucleotides, less than 7 nucleotides, less than 8 nucleotides, less than 9 nucleotides, less than 10 nucleotides, less than 11 nucleotides, less than 12 nucleotides, less than 13 nucleotides, less than 14 nucleotides, less than 15 nucleotides, less than 16 nucleotides, less than 17 nucleotides, less than 18 nucleotides, less than 19 nucleotides, less than 20 nucleotides, less than 21 nucleotides, less than 22 nucleotides, less than 23 nucleotides, less than 24 nucleotides, less than 25 nucleotides, less than 26 nucleotides, less than 27 nucleotides, less than 28 nucleotides, less than 29 nucleotides, less than 30 nucleotides, or less than 31 nucleotides.

It is preferred that, when hybridized to nucleic acid molecules in a nucleic acid sample, the primers hybridize at intervals that allow efficient amplification. This generally

can be accomplished by using a number of primers in the amplification reaction such that the primers collectively will be complementary to sequence in the nucleic acid sample at desired intervals. Thus, for example, a single 6 base primer will be complementary, on average, to a sequence once every 4096 nucleotides, two 6 base primers will be

5      complementary, on average, to a sequence once every 2048 nucleotides, three 6 base primers will be complementary, on average, to a sequence once every 1024 nucleotides, four 6 base primers will be complementary, on average, to a sequence once every 512 nucleotides, five 6 base primers will be complementary, on average, to a sequence once every 256 nucleotides, six 6 base primers will be complementary, on average, to a sequence

10     once every 128 nucleotides, seven 6 base primers will be complementary, on average, to a sequence once every 64 nucleotides, eight 6 base primers will be complementary, on average, to a sequence once every 32 nucleotides, nine 6 base primers will be complementary, on average, to a sequence once every 16 nucleotides, ten 6 base primers will be complementary, on average, to a sequence once every 8 nucleotides, and so on.

15     Four 8 base primers will be complementary, on average, to a sequence once every 8192 nucleotides, five 8 base primers will be complementary, on average, to a sequence once every 4096 nucleotides, six 8 base primers will be complementary, on average, to a sequence once every 1024 nucleotides, seven 8 base primers will be complementary, on average, to a sequence once every 512 nucleotides, eight 8 base primers will be

20     complementary, on average, to a sequence once every 256 nucleotides, nine 8 base primers will be complementary, on average, to a sequence once every 128 nucleotides, ten 8 base primers will be complementary, on average, to a sequence once every 64 nucleotides, eleven 8 base primers will be complementary, on average, to a sequence once every 32 nucleotides, twelve 8 base primers will be complementary, on average, to a sequence once

25     every 16 nucleotides, thirteen 8 base primers will be complementary, on average, to a sequence once every 8 nucleotides, and so on.

The primers can also be complementary to a sequence that occurs, on average, every 5,000 nucleotides or less, every 4,000 nucleotides or less, every 3,000 nucleotides or less, every 2,500 nucleotides or less, every 2,000 nucleotides or less, every 1,500 nucleotides or

30     less, every 1,000 nucleotides or less, every 900 nucleotides or less, every 800 nucleotides or less, every 700 nucleotides or less, every 600 nucleotides or less, every 500 nucleotides or less, every 400 nucleotides or less, every 300 nucleotides or less, every 200 nucleotides or

less, every 100 nucleotides or less, or every 50 nucleotides or less, on average, in the nucleic acid molecules of the nucleic acid sample.

These distances assume a random distribution of sequences, which is approximately true for nucleic acid sample of high complexity, such as genomic nucleic acid samples.

5      These distances are derived from the relationship $4^N$, where N is the number of bases in the primer. The distances can be affected by, for example, the G+C percentage of nucleotides in the nucleic acid sample since G+C percentages other than 50% will have altered distributions of specific nucleotides sequences. Further, the lower the sequence complexity of the nucleic acid sample, the more likely the distribution of specific nucleotide sequences

10     will be altered. However, these effects should not greatly affect the amplification results. The use of shorter primers will minimize these effects. Where the G+C percentage of the nucleic acid sample is other than 50%, primers can be chosen and/or designed that have a similar G+C percentage, either in each primer or collectively among the primers used for amplification.

15     The optimal interval or separation distance between primer complementary sequences (and thus, the optimum number of primers) will not be the same for all DNA polymerases, because this parameter is dependent on the net polymerization rate. A processive DNA polymerase will have a characteristic polymerization rate which may range from 5 to 300 nucleotides per second, and may be influenced by the presence or absence of

20     accessory ssDNA binding proteins and helicases. In the case of a non-processive polymerase, the net polymerization rate will depend on the enzyme concentration, because at higher concentrations there are more re-initiation events and thus the net polymerization rate will be increased. An example of a processive polymerase is φ29 DNA polymerase, which proceeds at 50 nucleotides per second. An example of a non-processive polymerase

25     is Vent exo(-) DNA polymerase, which will give effective polymerization rates of 4 nucleotides per second at low concentration, or 16 nucleotides per second at higher concentrations.

To obtain an optimal yield in the disclosed method, the number of primers and their composition can be adjusted to suit the polymerase being used. Use of one or a few primers

30     is preferred when using a polymerase with a rapid polymerization rate. Use of more primers is preferred when using a polymerase with a slower polymerization rate. The following assay can be used to determine optimal spacing with any polymerase. The assay uses some combination of one, two, three, four, five, six, seven, eight, nine, ten, eleven,

twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, and twenty

primers. Each new primer reduces the average distance between complementary sequences

in the nucleic acids to be amplified. The number of primers can be varied systematically

between a range of primer numbers (the average distance between priming sites varies with

5      the number of primers used). A series of reactions can be performed in which the same

nucleic acid sample is amplified using the different numbers of primers. The number of

primers that produces the highest experimental yield of DNA and/or the highest quality of

amplified product is the optimal primer number for the specific DNA polymerase, or DNA

polymerase plus accessory protein combination being used.

10          DNA replication initiated at the sites in nucleic acid molecules where the primers

hybridize will extend to and displace strands being replicated from primers hybridized at

adjacent sites. Displacement of an adjacent strand makes it available for hybridization to

another primer and subsequent initiation of another round of replication. This process is

referred to herein as strand displacement replication.

15          Any desired number of primers of different nucleotide sequence can be used, but use

of one or a few primers is preferred. The amplification reaction can be performed with, for

example, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen,

fourteen, fifteen, sixteen, or seventeen primers. More primers can be used. There is no

fundamental upper limit to the number of primers that can be used. However, the use of

20     fewer primers is preferred. When multiple primers are used, the primers should each have a

different specific nucleotide sequence.

The amplification reaction can be performed with a single primer and, for example,

with no additional primers, with 1 additional primer, with 2 additional primers, with 3

additional primers, with 4 additional primers, with 5 additional primers, with 6 additional

25     primers, with 7 additional primers, with 8 additional primers, with 9 additional primers,

with 10 additional primers, with 11 additional primers, with 12 additional primers, with 13

additional primers, with 14 additional primers, with 15 additional primers, with 16

additional primers, with 17 additional primers, with 18 additional primers, with 19

additional primers, with 20 additional primers, with 21 additional primers, with 22

30     additional primers, with 23 additional primers, with 24 additional primers, with 25

additional primers, with 26 additional primers, with 27 additional primers, with 28

additional primers, with 29 additional primers, with 30 additional primers, with 31

additional primers, with 32 additional primers, with 33 additional primers, with 34

36

additional primers, with 35 additional primers, with 36 additional primers, with 37

additional primers, with 38 additional primers, with 39 additional primers, with 40

additional primers, with 41 additional primers, with 42 additional primers, with 43

additional primers, with 44 additional primers, with 45 additional primers, with 46

5   additional primers, with 47 additional primers, with 48 additional primers, with 49

additional primers, with 50 additional primers, with 51 additional primers, with 52

additional primers, with 53 additional primers, with 54 additional primers, with 55

additional primers, with 56 additional primers, with 57 additional primers, with 58

additional primers, with 59 additional primers, with 60 additional primers, with 61

10  additional primers, with 62 additional primers, with 63 additional primers, with 64

additional primers, with 65 additional primers, with 66 additional primers, with 67

additional primers, with 68 additional primers, with 69 additional primers, with 70

additional primers, with 71 additional primers, with 72 additional primers, with 73

additional primers, with 74 additional primers, with 75 additional primers, with 76

15  additional primers, with 77 additional primers, with 78 additional primers, with 79

additional primers, with 80 additional primers, with 81 additional primers, with 82

additional primers, with 83 additional primers, with 84 additional primers, with 85

additional primers, with 86 additional primers, with 87 additional primers, with 88

additional primers, with 89 additional primers, with 90 additional primers, with 91

20  additional primers, with 92 additional primers, with 93 additional primers, with 94

additional primers, with 95 additional primers, with 96 additional primers, with 97

additional primers, with 98 additional primers, with 99 additional primers, with 100

additional primers, with 110 additional primers, with 120 additional primers, with 130

additional primers, with 140 additional primers, with 150 additional primers, with 160

25  additional primers, with 170 additional primers, with 180 additional primers, with 190

additional primers, with 200 additional primers, with 210 additional primers, with 220

additional primers, with 230 additional primers, with 240 additional primers, with 250

additional primers, with 260 additional primers, with 270 additional primers, with 280

additional primers, with 290 additional primers, with 300 additional primers, with 310

30  additional primers, with 320 additional primers, with 330 additional primers, with 340

additional primers, with 350 additional primers, with 360 additional primers, with 370

additional primers, with 380 additional primers, with 390 additional primers, with 400

additional primers, with 410 additional primers, with 420 additional primers, with 430

additional primers, with 440 additional primers, with 450 additional primers, with 460 additional primers, with 470 additional primers, with 480 additional primers, with 490 additional primers, with 500 additional primers, with 550 additional primers, with 600 additional primers, with 650 additional primers, with 700 additional primers, with 750

5    additional primers, with 800 additional primers, with 850 additional primers, with 900 additional primers, with 950 additional primers, with 1,000 additional primers, with 1,100 additional primers, with 1,200 additional primers, with 1,300 additional primers, with 1,400 additional primers, with 1,500 additional primers, with 1,600 additional primers, with 1,700 additional primers, with 1,800 additional primers, with 1,900 additional primers, with 2,000

10   additional primers, with 2,100 additional primers, with 2,200 additional primers, with 2,300 additional primers, with 2,400 additional primers, with 2,500 additional primers, with 2,600 additional primers, with 2,700 additional primers, with 2,800 additional primers, with 2,900 additional primers, with 3,000 additional primers, with 3,500 additional primers, or with 4,000 additional primers.

15         The amplification reaction can be performed with a single primer and, for example, with no additional primers, with fewer than 2 additional primers, with fewer than 3 additional primers, with fewer than 4 additional primers, with fewer than 5 additional primers, with fewer than 6 additional primers, with fewer than 7 additional primers, with fewer than 8 additional primers, with fewer than 9 additional primers, with fewer than 10

20   additional primers, with fewer than 11 additional primers, with fewer than 12 additional primers, with fewer than 13 additional primers, with fewer than 14 additional primers, with fewer than 15 additional primers, with fewer than 16 additional primers, with fewer than 17 additional primers, with fewer than 18 additional primers, with fewer than 19 additional primers, with fewer than 20 additional primers, with fewer than 21 additional primers, with

25   fewer than 22 additional primers, with fewer than 23 additional primers, with fewer than 24 additional primers, with fewer than 25 additional primers, with fewer than 26 additional primers, with fewer than 27 additional primers, with fewer than 28 additional primers, with fewer than 29 additional primers, with fewer than 30 additional primers, with fewer than 31 additional primers, with fewer than 32 additional primers, with fewer than 33 additional

30   primers, with fewer than 34 additional primers, with fewer than 35 additional primers, with fewer than 36 additional primers, with fewer than 37 additional primers, with fewer than 38 additional primers, with fewer than 39 additional primers, with fewer than 40 additional primers, with fewer than 41 additional primers, with fewer than 42 additional primers, with

fewer than 43 additional primers, with fewer than 44 additional primers, with fewer than 45 additional primers, with fewer than 46 additional primers, with fewer than 47 additional primers, with fewer than 48 additional primers, with fewer than 49 additional primers, with fewer than 50 additional primers, with fewer than 51 additional primers, with fewer than 52 additional primers, with fewer than 53 additional primers, with fewer than 54 additional primers, with fewer than 55 additional primers, with fewer than 56 additional primers, with fewer than 57 additional primers, with fewer than 58 additional primers, with fewer than 59 additional primers, with fewer than 60 additional primers, with fewer than 61 additional primers, with fewer than 62 additional primers, with fewer than 63 additional primers, with fewer than 64 additional primers, with fewer than 65 additional primers, with fewer than 66 additional primers, with fewer than 67 additional primers, with fewer than 68 additional primers, with fewer than 69 additional primers, with fewer than 70 additional primers, with fewer than 71 additional primers, with fewer than 72 additional primers, with fewer than 73 additional primers, with fewer than 74 additional primers, with fewer than 75 additional primers, with fewer than 76 additional primers, with fewer than 77 additional primers, with fewer than 78 additional primers, with fewer than 79 additional primers, with fewer than 80 additional primers, with fewer than 81 additional primers, with fewer than 82 additional primers, with fewer than 83 additional primers, with fewer than 84 additional primers, with fewer than 85 additional primers, with fewer than 86 additional primers, with fewer than 87 additional primers, with fewer than 88 additional primers, with fewer than 89 additional primers, with fewer than 90 additional primers, with fewer than 91 additional primers, with fewer than 92 additional primers, with fewer than 93 additional primers, with fewer than 94 additional primers, with fewer than 95 additional primers, with fewer than 96 additional primers, with fewer than 97 additional primers, with fewer than 98 additional primers, with fewer than 99 additional primers, with fewer than 100 additional primers, with fewer than 110 additional primers, with fewer than 120 additional primers, with fewer than 130 additional primers, with fewer than 140 additional primers, with fewer than 150 additional primers, with fewer than 160 additional primers, with fewer than 170 additional primers, with fewer than 180 additional primers, with fewer than 190 additional primers, with fewer than 200 additional primers, with fewer than 210 additional primers, with fewer than 220 additional primers, with fewer than 230 additional primers, with fewer than 240 additional primers, with fewer than 250 additional primers, with fewer than 260 additional primers, with fewer than 270 additional primers, with fewer than 280 additional primers, with fewer

than 290 additional primers, with fewer than 300 additional primers, with fewer than 310 additional primers, with fewer than 320 additional primers, with fewer than 330 additional primers, with fewer than 340 additional primers, with fewer than 350 additional primers, with fewer than 360 additional primers, with fewer than 370 additional primers, with fewer

5   than 380 additional primers, with fewer than 390 additional primers, with fewer than 400 additional primers, with fewer than 410 additional primers, with fewer than 420 additional primers, with fewer than 430 additional primers, with fewer than 440 additional primers, with fewer than 450 additional primers, with fewer than 460 additional primers, with fewer than 470 additional primers, with fewer than 480 additional primers, with fewer than 490

10   additional primers, with fewer than 500 additional primers, with fewer than 550 additional primers, with fewer than 600 additional primers, with fewer than 650 additional primers, with fewer than 700 additional primers, with fewer than 750 additional primers, with fewer than 800 additional primers, with fewer than 850 additional primers, with fewer than 900 additional primers, with fewer than 950 additional primers, with fewer than 1,000 additional

15   primers, with fewer than 1,100 additional primers, with fewer than 1,200 additional primers, with fewer than 1,300 additional primers, with fewer than fewer than 1,400 additional primers, with fewer than 1,500 additional primers, with fewer than 1,600 additional primers, with fewer than 1,700 additional primers, with fewer than 1,800 additional primers, with fewer than 1,900 additional primers, with fewer than 2,000 additional primers, with fewer

20   than 2,100 additional primers, with fewer than 2,200 additional primers, with fewer than 2,300 additional primers, with fewer than 2,400 additional primers, with fewer than 2,500 additional primers, with fewer than 2,600 additional primers, with fewer than 2,700 additional primers, with fewer than 2,800 additional primers, with fewer than 2,900 additional primers, with fewer than 3,000 additional primers, with fewer than 3,500

25   additional primers, or with fewer than 4,000 additional primers.

The amplification reaction can be performed, for example, with fewer than 2 primers, with fewer than 3 primers, with fewer than 4 primers, with fewer than 5 primers, with fewer than 6 primers, with fewer than 7 primers, with fewer than 8 primers, with fewer than 9 primers, with fewer than 10 primers, with fewer than 11 primers, with fewer than 12

30   primers, with fewer than 13 primers, with fewer than 14 primers, with fewer than 15 primers, with fewer than 16 primers, with fewer than 17 primers, with fewer than 18 primers, with fewer than 19 primers, with fewer than 20 primers, with fewer than 21 primers, with fewer than 22 primers, with fewer than 23 primers, with fewer than 24

primers, with fewer than 25 primers, with fewer than 26 primers, with fewer than 27 primers, with fewer than 28 primers, with fewer than 29 primers, with fewer than 30 primers, with fewer than 31 primers, with fewer than 32 primers, with fewer than 33 primers, with fewer than 34 primers, with fewer than 35 primers, with fewer than 36

5      primers, with fewer than 37 primers, with fewer than 38 primers, with fewer than 39 primers, with fewer than 40 primers, with fewer than 41 primers, with fewer than 42 primers, with fewer than 43 primers, with fewer than 44 primers, with fewer than 45 primers, with fewer than 46 primers, with fewer than 47 primers, with fewer than 48 primers, with fewer than 49 primers, with fewer than 50 primers, with fewer than 51

10     primers, with fewer than 52 primers, with fewer than 53 primers, with fewer than 54 primers, with fewer than 55 primers, with fewer than 56 primers, with fewer than 57 primers, with fewer than 58 primers, with fewer than 59 primers, with fewer than 60 primers, with fewer than 61 primers, with fewer than 62 primers, with fewer than 63 primers, with fewer than 64 primers, with fewer than 65 primers, with fewer than 66

15     primers, with fewer than 67 primers, with fewer than 68 primers, with fewer than 69 primers, with fewer than 70 primers, with fewer than 71 primers, with fewer than 72 primers, with fewer than 73 primers, with fewer than 74 primers, with fewer than 75 primers, with fewer than 76 primers, with fewer than 77 primers, with fewer than 78 primers, with fewer than 79 primers, with fewer than 80 primers, with fewer than 81

20     primers, with fewer than 82 primers, with fewer than 83 primers, with fewer than 84 primers, with fewer than 85 primers, with fewer than 86 primers, with fewer than 87 primers, with fewer than 88 primers, with fewer than 89 primers, with fewer than 90 primers, with fewer than 91 primers, with fewer than 92 primers, with fewer than 93 primers, with fewer than 94 primers, with fewer than 95 primers, with fewer than 96

25     primers, with fewer than 97 primers, with fewer than 98 primers, with fewer than 99 primers, with fewer than 100 primers, with fewer than 110 primers, with fewer than 120 primers, with fewer than 130 primers, with fewer than 140 primers, with fewer than 150 primers, with fewer than 160 primers, with fewer than 170 primers, with fewer than 180 primers, with fewer than 190 primers, with fewer than 200 primers, with fewer than 210

30     primers, with fewer than 220 primers, with fewer than 230 primers, with fewer than 240 primers, with fewer than 250 primers, with fewer than 260 primers, with fewer than 270 primers, with fewer than 280 primers, with fewer than 290 primers, with fewer than 300 primers, with fewer than 310 primers, with fewer than 320 primers, with fewer than 330

primers, with fewer than 340 primers, with fewer than 350 primers, with fewer than 360 primers, with fewer than 370 primers, with fewer than 380 primers, with fewer than 390 primers, with fewer than 400 primers, with fewer than 410 primers, with fewer than 420 primers, with fewer than 430 primers, with fewer than 440 primers, with fewer than 450

5      primers, with fewer than 460 primers, with fewer than 470 primers, with fewer than 480 primers, with fewer than 490 primers, with fewer than 500 primers, with fewer than 550 primers, with fewer than 600 primers, with fewer than 650 primers, with fewer than 700 primers, with fewer than 750 primers, with fewer than 800 primers, with fewer than 850 primers, with fewer than 900 primers, with fewer than 950 primers, with fewer than 1,000

10     primers, with fewer than 1,100 primers, with fewer than 1,200 primers, with fewer than 1,300 primers, with fewer than fewer than 1,400 primers, with fewer than 1,500 primers, with fewer than 1,600 primers, with fewer than 1,700 primers, with fewer than 1,800 primers, with fewer than 1,900 primers, with fewer than 2,000 primers, with fewer than 2,100 primers, with fewer than 2,200 primers, with fewer than 2,300 primers, with fewer

15     than 2,400 primers, with fewer than 2,500 primers, with fewer than 2,600 primers, with fewer than 2,700 primers, with fewer than 2,800 primers, with fewer than 2,900 primers, with fewer than 3,000 primers, with fewer than 3,500 primers, or with fewer than 4,000 primers.

          The primers used in the disclosed method can be selected and/or designed to have

20     certain desirable features and functional characteristics. The goal of primer selection and primer design can be obtaining better amplification results. For example, particular primers can be selected that result in the highest amplification yield (that is, the highest amount of increase in the amount of nucleic acid), the best locus or sequence representation in the amplified nucleic acid (that is, the closest to 100% locus or sequence representation for loci

25     and sequences of interest), and/or the lowest amplification bias. This can be determined by testing particular primers in amplification reactions using a nucleic acid sample of interest. Different primers may produce optimal results for different nucleic acid samples. However, the primer number and primer composition principles described herein will generally produce good amplification results on nearly every nucleic acid sample. This broad-based

30     usefulness of the disclosed primers and method is a useful feature of the disclosed primers and method.

          Primers that produce amplification products of a desired quality are referred to herein as broad coverage primers. In general, a broad coverage primer (or primers, when

used together) can produce a locus representation of at least 10% for at least 5 different loci, a sequence representation of at least 10% for at least 5 different target sequences, an amplification bias of less than 50-fold, an amplification bias of less than 50-fold for at least 5 different loci, and/or an amplification bias of less than 50-fold for at least 5 different

5    target sequences. Primers can also produce, for example, a locus representation of at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% for at least 5 different loci. Primers can also produce, for example, a locus representation of at least 10% for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9

10   different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

15          Primers can also produce, for example, a sequence representation of at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% for at least 5 different target sequences. Primers can also produce, for example, a sequence representation of at least 10% for at least 6 different target sequences, at least 7 different target sequences, at

20   least 8 different target sequences, at least 9 different target sequences, at least 10 different target sequences, at least 11 different target sequences, at least 12 different target sequences, at least 13 different target sequences, at least 14 different target sequences, at least 15 different target sequences, at least 16 different target sequences, at least 17 different target sequences, at least 18 different target sequences, at least 19 different target sequences, at

25   least 20 different target sequences, at least 25 different target sequences, at least 30 different target sequences, at least 40 different target sequences, at least 50 different target sequences, at least 75 different target sequences, or at least 100 different target sequences.

          Primers can also produce, for example, an amplification bias of less than 45-fold, less than 40-fold, less than 35-fold, less than 30-fold, less than 25-fold, less than 20-fold,

30   less than 19-fold, less than 18-fold, less than 17-fold, less than 16-fold, less than 15-fold, less than 14-fold, less than 13-fold, less than 12-fold, less than 11-fold, less than 10-fold, less than 9-fold, less than 8-fold, less than 7-fold, less than 6-fold, less than 5-fold, or less than 4-fold. Primers can also produce, for example, an amplification bias of less than 50-

fold for at least 5 different loci, for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci. Primers can also produce, for example, an amplification bias of less than 50-fold for at least 5 different target sequences, for at least 6 different target sequences, at least 7 different target sequences, at least 8 different target sequences, at least 9 different target sequences, at least 10 different target sequences, at least 11 different target sequences, at least 12 different target sequences, at least 13 different target sequences, at least 14 different target sequences, at least 15 different target sequences, at least 16 different target sequences, at least 17 different target sequences, at least 18 different target sequences, at least 19 different target sequences, at least 20 different target sequences, at least 25 different target sequences, at least 30 different target sequences, at least 40 different target sequences, at least 50 different target sequences, at least 75 different target sequences, or at least 100 different target sequences.

These results can be over a variety of nucleic acid samples, for some selected types of nucleic acid samples, or for a specific type of nucleic acid sample. Thus, a broad coverage primer can be a broad coverage primer when used for, for example, a specific type of nucleic acid sample, when used for selected types of nucleic acid samples, or when used for a variety of nucleic acid samples or nucleic acid samples in general. Thus, the designation broad coverage primer is generally dependent on the nucleic acid sample involved and can also depend on the DNA polymerase used and the conditions used.

Regarding primer selection and design, as described above and elsewhere herein, the primers can be designed (in length and number of primers used) to hybridize at certain intervals, on average, in the nucleotide sequences in the nucleic acid sample. Distribution of primer complement sites can also be achieved by choosing primer sequences that are complementary to sequences that are repeated many times in the nucleic acid sample. Such sequences include classic repeat sequences, such as microsatellite sequences, minisatellite sequences, satellite sequences, transposon sequences, ribosomal RNA sequences, short interspersed nuclear elements (SINEs), or long interspersed nuclear elements (LINEs); and functional consensus sequences, such as promoter sequences, enhancer sequences, silencer

sequences, upstream regulatory element sequences, transcription termination site sequences, transposon regulatory sequences, ribosomal RNA regulatory sequences, or polyadenylation site sequences. For example, the primer sequence can be chosen to be complementary to a sequence in an Alu repeat sequence. As a specific example, the primer can have one of the

5      sequences AGTGGG or AGAGAG; one of the sequences AGCCGG, AGTAGG, or AGTTGG; one of the sequences AGGCGG, AGTGGG, AGGGAG, or AGTGAG; one of the sequences AGTGGG, AGCCAG, AGTTAG, AGTCAG, or AGACAG; one of the sequences AGAGGG, AGGCAG, AGCCAG, AGTCAG, or AGACAG; one of the sequences AGTAGG, AGGTGG, AGGCAG, AGACAG, or AGTGAG; AGGAGG,

10    AGAGGG, AGGGAG, AGTCAG, or AGCGAG; or one of the sequences CGGTGG, TCACGC, CGAGCG, GCGTGG, ACTCGG, AATCGC, CGGAGG, CCGAGA, GATCGC, AGAGCG, AGCGAG, or ACTCCG. Multiple primers used in a reaction can have different sequences that are, for example, complementary to different sequences in an Alu repeat sequence. As a specific example, each primer has a different one of the

15    sequences AGTGGG or AGAGAG; a different one of the sequences AGCCGG, AGTAGG, or AGTTGG; a different one of the sequences AGGCGG, AGTGGG, AGGGAG, or AGTGAG; a different one of the sequences AGTGGG, AGCCAG, AGTTAG, AGTCAG, or AGACAG; a different one of the sequences AGAGGG, AGGCAG, AGCCAG, AGTCAG, or AGACAG; a different one of the sequences AGTAGG, AGGTGG,

20    AGGCAG, AGACAG, or AGTGAG; AGGAGG, AGAGGG, AGGGAG, AGTCAG, or AGCGAG; or a different one of the sequences CGGTGG, TCACGC, CGAGCG, GCGTGG, ACTCGG, AATCGC, CGGAGG, CCGAGA, GATCGC, AGAGCG, AGCGAG, or ACTCCG.

       The nucleotide sequence and composition of the primers used can also be chosen to

25    optimize amplification. For example, the G+C percentage of the primers can be chosen based on the G+C percentage of the nucleic acid sample to be amplified. The primer can have, for example, a G+C percentage within 20%, within 15%, within 10%, within 9%, within 8%, within 7%, within 6%, within 5%, within 4%, within 3%, within 2%, or within 1% of the G+C percentage of the nucleic acid sample. As used herein, G+C percentage

30    refers to the percent of total nucleotides that are either guanosine (G) residues or cytidine (C) residues in a given nucleic acid molecule, nucleic acid sequence, nucleic acid sample, or other nucleic acid composition.

The primers can also have other characteristics that can, for example, increase amplification yield and reduce production of artifacts or artifactual amplification. For example, generation of primer dimer artifacts can be reduced by designing primers to avoid 3' end sequences that are complementary, either between primers or within the same primer. Such sequences to be avoided can be referred to as inter-complementary 3' ends. A useful measure of a primer's ability to avoid artifactual amplification is the lack or relative insignificance of amplification (that is, nucleic acid produced) when the primer is used in an amplification reaction without a nucleic acid sample.

The disclosed primers can have one or more modified nucleotides. Such primers are referred to herein as modified primers. Modified primers have several advantages. First, some forms of modified primers, such as RNA/ 2'-O-methyl RNA chimeric primers, have a higher melting temperature (Tm) than DNA primers. This increases the stability of primer hybridization and will increase strand invasion by the primers. This will lead to more efficient priming. Also, since the primers are made of RNA, they will be exonuclease resistant. Such primers, if tagged with minor groove binders at their 5' end, will also have better strand invasion of the template dsDNA. In addition, RNA primers can also be very useful for WGA from biological samples such as cells or tissue. Since the biological samples contain endogenous RNA, this RNA can be degraded with RNase to generate a pool of random oligomers, which can then be used to prime the polymerase for amplification of the DNA. This eliminates any need to add primers to the reaction. Alternatively, DNase digestion of biological samples can generate a pool of DNA oligo primers for RNA dependent DNA amplification.

Chimeric primers can also be used. Chimeric primers are primers having at least two types of nucleotides, such as both deoxyribonucleotides and ribonucleotides, ribonucleotides and modified nucleotides, or two different types of modified nucleotides. One form of chimeric primer is peptide nucleic acid/nucleic acid primers. For example, 5'-PNA-DNA-3' or 5'-PNA-RNA-3' primers may be used for more efficient strand invasion and polymerization invasion. The DNA and RNA portions of such primers can have random or degenerate sequences. Other forms of chimeric primers are, for example, 5'- (2'-O-Methyl) RNA-RNA-3' or 5'- (2'-O-Methyl) RNA-DNA-3'.

Many modified nucleotides (nucleotide analogs) are known and can be used in oligonucleotides. A nucleotide analog is a nucleotide which contains some type of modification to either the base, sugar, or phosphate moieties. Modifications to the base

moiety would include natural and synthetic modifications of A, C, G, and T/U as well as different purine or pyrimidine bases, such as uracil-5-yl, hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl. A modified base includes but is not limited to 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl

5    and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and

10   other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Additional base modifications can be found for example in U.S. Pat. No. 3,687,808, Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and Sanghvi, Y. S., Chapter 15, Antisense Research and Applications, pages 289-302,

15   Crooke, S. T. and Lebleu, B. ed., CRC Press, 1993. Certain nucleotide analogs, such as 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine can increase the stability of duplex formation. Other modified bases are those that function as universal bases. Universal bases include 3-nitropyrrole and 5-

20   nitroindole. Universal bases substitute for the normal bases but have no bias in base pairing. That is, universal bases can base pair with any other base. Primers composed, either in whole or in part, of nucleotides with universal bases are useful for reducing or eliminating amplification bias against repeated sequences in a target sample. This would be useful, for example, where a loss of sequence complexity in the amplified products is

25   undesirable. Base modifications often can be combined with for example a sugar modification, such as 2'-O-methoxyethyl, to achieve unique properties such as increased duplex stability. There are numerous United States patents such as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, which

30   detail and describe a range of base modifications. Each of these patents is herein incorporated by reference.

Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety would include natural modifications of the ribose and

deoxyribose as well as synthetic modifications. Sugar modifications include but are not limited to the following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C1 to C10, alkyl or C2 to C10 alkenyl and

5    alkynyl. 2' sugar modifications also include but are not limited to -O[(CH$_2$)n O]m CH$_3$, - O(CH$_2$)n OCH$_3$, -O(CH$_2$)n NH$_2$, -O(CH$_2$)n CH$_3$, -O(CH$_2$)n -ONH$_2$, and - O(CH$_2$)nON[(CH$_2$)n CH$_3$)]$_2$, where n and m are from 1 to about 10.

Other modifications at the 2' position include but are not limited to: C1 to C10 lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH$_3$, OCN, Cl,

10   Br, CN, CF$_3$, OCF$_3$, SOCH$_3$, SO$_2$ CH$_3$, ONO$_2$, NO$_2$, N$_3$, NH$_2$, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar

15   modifications may also be made at other positions on the sugar, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Modified sugars would also include those that contain modifications at the bridging ring oxygen, such as CH$_2$ and S. Nucleotide sugar analogs may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl

20   sugar. There are numerous United States patents that teach the preparation of such modified sugar structures such as 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety.

25   Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include but are not limited to those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates,

30   phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. It is understood that these phosphate or modified phosphate linkages between two nucleotides can be through a 3'-5' linkage or a 2'-5' linkage, and the linkage

can contain inverted polarity such as 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. Numerous United States patents teach how to make and use nucleotides containing modified phosphates and include but are not limited to, 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019;

5      5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

It is understood that nucleotide analogs need only contain a single modification, but may also contain multiple modifications within one of the moieties or between different

10     moieties.

Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize and hybridize to complementary nucleic acids in a Watson-Crick or Hoogsteen manner, but which are

15     linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

Nucleotide substitutes are nucleotides or nucleotide analogs that have had the phosphate moiety and/or sugar moieties replaced. Nucleotide substitutes do not contain a

20     standard phosphorus atom. Substitutes for the phosphate can be for example, short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone

25     backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH2 component parts. Numerous United States patents disclose how to make and use these types of phosphate replacements

30     and include but are not limited to 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289;

5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

It is also understood in a nucleotide substitute that both the sugar and the phosphate moieties of the nucleotide can be replaced, by for example an amide type linkage
5    (aminoethylglycine) (PNA). United States patents 5,539,082; 5,714,331; and 5,719,262 teach how to make and use PNA molecules, each of which is herein incorporated by reference. (See also Nielsen *et al.*, *Science* 254:1497-1500 (1991)).

Primers can be comprised of nucleotides and can be made up of different types of nucleotides or the same type of nucleotides. For example, one or more of the nucleotides in
10   a primer can be ribonucleotides, 2'-O-methyl ribonucleotides, or a mixture of ribonucleotides and 2'-O-methyl ribonucleotides; about 10% to about 50% of the nucleotides can be ribonucleotides, 2'-O-methyl ribonucleotides, or a mixture of ribonucleotides and 2'-O-methyl ribonucleotides; about 50% or more of the nucleotides can be ribonucleotides, 2'-O-methyl ribonucleotides, or a mixture of ribonucleotides and 2'-O-
15   methyl ribonucleotides; or all of the nucleotides are ribonucleotides, 2'-O-methyl ribonucleotides, or a mixture of ribonucleotides and 2'-O-methyl ribonucleotides. The nucleotides can be comprised of bases (that is, the base portion of the nucleotide) and can (and normally will) comprise different types of bases. For example, one or more of the bases can be universal bases, such as 3-nitropyrrole or 5-nitroindole; about 10% to about
20    50% of the bases can be universal bases; about 50% or more of the bases can be universal bases; or all of the bases can be universal bases.

Primers may, but need not, also contain additional sequence at the 5' end of the primer that is not complementary to the target sequence. This sequence is referred to as the non-complementary portion of the primer. The non-complementary portion of the primer, if
25   present, serves to facilitate strand displacement during DNA replication. The non-complementary portion of the primer can also include a functional sequence such as a promoter for an RNA polymerase. The non-complementary portion of a primer may be any length, but is generally 1 to 100 nucleotides long, and preferably 4 to 8 nucleotides long. The use of a non-complementary portion is not preferred when random or partially random
30   primers are used for whole genome amplification.

It is specifically contemplated that primers having random or degenerate sequence can be excluded from use in the disclosed method. It is also specifically contemplated that use of conditions that allow or are compatible with substantial, significant or notable

50

mismatch hybridization of the primers to nucleic acid molecules being amplified can be excluded. As used herein, substantial mismatch hybridization of a primer refers to hybridization where 90% or more of the primer nucleotides are unpaired to nucleotides in the hybridization partner. Significant mismatch hybridization of a primer refers to

5      hybridization where 50% or more of the primer nucleotides are unpaired to nucleotides in the hybridization partner. Notable mismatch hybridization of a primer refers to hybridization where 10% or more of the primer nucleotides are unpaired to nucleotides in the hybridization partner. Choosing conditions that avoid or that are not compatible with substantial or significant or notable mismatch hybridization of the primers emphasizes the

10     use of specific or substantially specific hybridization of the primers in the disclosed method.

        As used herein, conditions compatible with a given level of mismatch hybridization refer to conditions that would result in a notable fraction or more of hybridizations to be at the given level. Conditions that are not compatible with a given level of mismatch hybridization refer to conditions that would not result in a notable fraction of hybridizations

15     to be at the given level. Conditions that allow a given level of mismatch hybridization refer to conditions that would result in a notable fraction or more of hybridizations to be at the given level. Conditions that do not allow a given level of mismatch hybridization refer to conditions that would not result in a notable fraction of hybridizations to be at the given level. In this regard, it is understood that conditions that theoretically would or would not

20     produce a given level of hybridization will not prevent some transient or rare mismatch hybridizations.

        An important factor for conditions that do or do not allow, or that are or are not compatible with, a given level of mismatch hybridization is the temperature at which the amplification is carried out. Thus, for example, a temperature significantly below the

25     melting temperature of a primer generally would allow a higher level of mismatch hybridization by that primer than a temperature closer to its melting temperature because hybrids involving only some of the nucleotides in the primer would be stable at the lower temperature. In this way, the reaction temperature (that is, the temperature at which the nucleic acid sample, primer and DNA polymerase are incubated for amplification) affects

30     the level of mismatch hybridization and the intervals at which primers will hybridize to nucleotide sequences in the nucleic acid sample.

        To make use of primer specificity in the disclosed method, the primers can be designed (or, conversely, the incubation temperature can be chosen) to reduce the level of

51

mismatch hybridization. In general, this can involve use of lower incubation temperatures for shorter primers and higher incubation temperatures for longer primers. As deemed suitable and desirable, the primers can be designed for use at, and/or the amplification reaction can be incubated at 20°C, 21°C, 22°C, 23°C, 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, 5    30°C, 31°C, 32°C, 33°C, 34°C, 35°C, 36°C, 37°C, 38°C, 39°C, 40°C, 41°C, 42°C, 43°C, 44°C, 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 67°C, 68°C, 69°C, 70°C, 71°C, 72°C, 73°C, 74°C, 75°C, 76°C, 77°C, 78°C, 79°C, or 80°C. The primers can be designed for use at, and/or the amplification reaction can be incubated at less than 21°C, less than 10    22°C, less than 23°C, less than 24°C, less than 25°C, less than 26°C, less than 27°C, less than 28°C, less than 29°C, less than 30°C, less than 31°C, less than 32°C, less than 33°C, less than 34°C, less than 35°C, less than 36°C, less than 37°C, less than 38°C, less than 39°C, less than 40°C, less than 41°C, less than 42°C, less than 43°C, less than 44°C, less than 45°C, less than 46°C, less than 47°C, less than 48°C, less than 49°C, less than 50°C, 15    less than 51°C, less than 52°C, less than 53°C, less than 54°C, less than 55°C, less than 56°C, less than 57°C, less than 58°C, less than 59°C, less than 60°C, less than 61°C, less than 62°C, less than 63°C, less than 64°C, less than 65°C, less than 66°C, less than 67°C, less than 68°C, less than 69°C, less than 70°C, less than 71°C, less than 72°C, less than 73°C, less than 74°C, less than 75°C, less than 76°C, less than 77°C, less than 78°C, less 20    than 79°C, or less than 80°C.

## 1. Primers for Whole Genome Strand Displacement Amplification

In the case of whole genome strand displacement amplification, it is preferred that a set of primers having random or partially random nucleotide sequences be used. In a nucleic acid sample of significant or substantial complexity, which is the preferred target 25    sequence for WGSDA, specific nucleic acid sequences present in the sample need not be known and the primers need not be designed to be complementary to any particular sequence. Rather, the complexity of the nucleic acid sample results in a large number of different hybridization target sequences in the sample which will be complementary to various primers of random or partially random sequence. The complementary portion of 30    primers for use in WGSDA can be fully randomized, have only a portion that is randomized, or be otherwise selectively randomized. Sets of primers having random or partially random sequences can be synthesized using standard techniques by allowing the addition of any nucleotide at each position to be randomized. It is also preferred that the

sets of primers are composed of primers of similar length and/or hybridization characteristics.

The number of random base positions in the complementary portion of primers are preferably from 20% to 100% of the total number of nucleotides in the complementary portion of the primers. More preferably the number of random base positions are from 30% to 100% of the total number of nucleotides in the complementary portion of the primers. Most preferably the number of random base positions are from 50% to 100% of the total number of nucleotides in the complementary portion of the primers. Sets of primers having random or partially random sequences can be synthesized using standard techniques by allowing the addition of any nucleotide at each position to be randomized. It is also preferred that the sets of primers are composed of primers of similar length and/or hybridization characteristics.

## 2. Primers for Multiple Strand Displacement Amplification

In the case of multiple strand displacement amplification, the complementary portion of each primer is designed to be complementary to the hybridization target in the target sequence. In a set of primers, it is preferred that the complementary portion of each primer be complementary to a different portion of the target sequence. It is more preferred that the primers in the set be complementary to adjacent sites in the target sequence. It is also preferred that such adjacent sites in the target sequence are also adjacent to the amplification target in the target sequence.

It is preferred that, when hybridized to a target sequence, the primers in a set of primers are separated from each other. It is preferred that, when hybridized, the primers in a set of primers are separated from each other by at least 5 bases. It is more preferred that, when hybridized, the primers in a set of primers are separated from each other by at least 10 bases. It is still more preferred that, when hybridized, the primers in a set of primers are separated from each other by at least 20 bases. It is still more preferred that, when hybridized, the primers in a set of primers are separated from each other by at least 30 bases. It is still more preferred that, when hybridized, the primers in a set of primers are separated from each other by at least 40 bases. It is still more preferred that, when hybridized, the primers in a set of primers are separated from each other by at least 50 bases.

It is preferred that, when hybridized, the primers in a set of primers are separated from each other by no more than about 500 bases. It is more preferred that, when

hybridized, the primers in a set of primers are separated from each other by no more than about 400 bases. It is still more preferred that, when hybridized, the primers in a set of primers are separated from each other by no more than about 300 bases. It is still more preferred that, when hybridized, the primers in a set of primers are separated from each

5      other by no more than about 200 bases. Any combination of the preferred upper and lower limits of separation described above are specifically contemplated, including all intermediate ranges. The primers in a set of primers need not, when hybridized, be separated from each other by the same number of bases. It is preferred that, when hybridized, the primers in a set of primers are separated from each other by about the same

10     number of bases.

The optimal separation distance between primers will not be the same for all DNA polymerases, because this parameter is dependent on the net polymerization rate. A processive DNA polymerase will have a characteristic polymerization rate which may range from 5 to 300 nucleotides per second, and may be influenced by the presence or absence of

15     accessory ssDNA binding proteins and helicases. In the case of a non-processive polymerase, the net polymerization rate will depend on the enzyme concentration, because at higher concentrations there are more re-initiation events and thus the net polymerization rate will be increased. An example of a processive polymerase is $\phi$29 DNA polymerase, which proceeds at 50 nucleotides per second. An example of a non-processive polymerase

20     is Vent exo(-) DNA polymerase, which will give effective polymerization rates of 4 nucleotides per second at low concentration, or 16 nucleotides per second at higher concentrations.

To obtain an optimal yield in an MSDA reaction, the primer spacing is preferably adjusted to suit the polymerase being used. Long primer spacing is preferred when using a

25     polymerase with a rapid polymerization rate. Shorter primer spacing is preferred when using a polymerase with a slower polymerization rate. The following assay can be used to determine optimal spacing with any polymerase. The assay uses sets of primers, with each set made up of 5 left primers and 5 right primers. The sets of primers are designed to hybridize adjacent to the same target sequence with each of the different sets of primers

30     having a different primer spacing. The spacing is varied systematically between the sets of primers in increments of 25 nucleotides within the range of 25 nucleotides to 400 nucleotides (the spacing of the primers within each set is the same). A series of reactions are performed in which the same target sequence is amplified using the different sets of

54

primers. The spacing that produces the highest experimental yield of DNA is the optimal primer spacing for the specific DNA polymerase, or DNA polymerase plus accessory protein combination being used.

DNA replication initiated at the sites in the target sequence where the primers hybridize will extend to and displace strands being replicated from primers hybridized at adjacent sites. Displacement of an adjacent strand makes it available for hybridization to another primer and subsequent initiation of another round of replication. The region(s) of the target sequence to which the primers hybridize is referred to as the hybridization target of the target sequence.

A set of primers can include any desired number of primers of different nucleotide sequence. For MSDA, it is preferred that a set of primers include a plurality of primers. It is more preferred that a set of primers include 3 or more primers. It is still more preferred that a set of primers include 4 or more, 5 or more, 6 or more, or 7 or more primers. In general, the more primers used, the greater the level of amplification that will be obtained. There is no fundamental upper limit to the number of primers that a set of primers can have. However, for a given target sequence, the number of primers in a set of primers will generally be limited to the number of hybridization sites available in the target sequence. For example, if the target sequence is a 10,000 nucleotide DNA molecule and 20 nucleotide primers are used, there are 500 non-overlapping 20 nucleotide sites in the target sequence. Even more primers than this could be used if overlapping sites are either desired or acceptable. It is preferred that a set of primers include no more than about 300 primers. It is preferred that a set of primers include no more than about 200 primers. It is still more preferred that a set of primers include no more than about 100 primers. It is more preferred that a set of primers include no more than about 50 primers. It is most preferred that a set of primers include from 7 to about 50 primers. Any combination of the preferred upper and lower limits for the number of primers in a set of primers described above are specifically contemplated, including all intermediate ranges.

A preferred form of primer set for use in MSDA includes two sets of primers, referred to as a right set of primers and a left set of primers. The right set of primers and left set of primers are designed to be complementary to opposite strands of a target sequence. It is preferred that the complementary portions of the right set of primers are each complementary to the right hybridization target, and that each is complementary to a different portion of the right hybridization target. It is preferred that the complementary

portions of the left set of primers are each complementary to the left hybridization target, and that each is complementary to a different portion of the left hybridization target. The right and left hybridization targets flank opposite ends of the amplification target in a target sequence. It is preferred that a right set of primers and a left set of primers each include a

5      preferred number of primers as described above for a set of primers. Specifically, it is preferred that a right or left set of primers include a plurality of primers. It is more preferred that a right or left set of primers include 3 or more primers. It is still more preferred that a right or left set of primers include 4 or more, 5 or more, 6 or more, or 7 or more primers. It is preferred that a right or left set of primers include no more than about

10     200 primers. It is more preferred that a right or left set of primers include no more than about 100 primers. It is most preferred that a right or left set of primers include from 7 to about 100 primers. Any combination of the preferred upper and lower limits for the number of primers in a set of primers described above are specifically contemplated, including all intermediate ranges. It is also preferred that, for a given target sequence, the right set of

15     primers and the left set of primers include the same number of primers. It is also preferred that, for a given target sequence, the right set of primers and the left set of primers are composed of primers of similar length and/or hybridization characteristics.

        Where the target sequence(s) are present in mixed sample--for example, a nosocomial sample containing both human and non-human nucleic acids—the primers used

20     can be specific for the nucleic acids of interest. Thus, to detect pathogen (that is, non-human) nucleic acids in a patient sample, primers specific to pathogen nucleic acids can be used. If human nucleic acids are to be detected, then primers specific to human nucleic acids can be used. In this context, primers specific for particular target nucleic acids or target sequences or a particular class of target nucleic acids or target sequences refer to

25     primers that support amplification of the target nucleic acids and target sequences but do not support substantial amplification of non-target nucleic acids or sequences that are in the relevant sample.

### 3. Detection Tags

        The non-complementary portion of a primer can include sequences to be used to

30     further manipulate or analyze amplified sequences. An example of such a sequence is a detection tag, which is a specific nucleotide sequence present in the non-complementary portion of a primer. Detection tags have sequences complementary to detection probes. Detection tags can be detected using their cognate detection probes. Detection tags become

incorporated at the ends of amplified strands. The result is amplified DNA having detection
tag sequences that are complementary to the complementary portion of detection probes. If
present, there may be one, two, three, or more than three detection tags on a primer. It is
preferred that a primer have one, two, three or four detection tags. Most preferably, a

5      primer will have one detection tag. Generally, it is preferred that a primer have 10 detection
tags or less. There is no fundamental limit to the number of detection tags that can be
present on a primer except the size of the primer. When there are multiple detection tags,
they may have the same sequence or they may have different sequences, with each different
sequence complementary to a different detection probe. It is preferred that a primer contain

10     detection tags that have the same sequence such that they are all complementary to a single
detection probe. For some multiplex detection methods, it is preferable that primers contain
up to six detection tags and that the detection tag portions have different sequences such that
each of the detection tag portions is complementary to a different detection probe. A
similar effect can be achieved by using a set of primers where each has a single different

15     detection tag. The detection tags can each be any length that supports specific and stable
hybridization between the detection tags and the detection probe. For this purpose, a length
of 10 to 35 nucleotides is preferred, with a detection tag portion 15 to 20 nucleotides long
being most preferred.

### 4. Address Tag

20             Another example of a sequence that can be included in the non-complementary
portion of a primer is an address tag. An address tag has a sequence complementary to an
address probe. Address tags become incorporated at the ends of amplified strands. The
result is amplified DNA having address tag sequences that are complementary to the
complementary portion of address probes. If present, there may be one, or more than one,

25     address tag on a primer. It is preferred that a primer have one or two address tags. Most
preferably, a primer will have one address tag. Generally, it is preferred that a primer have
10 address tags or less. There is no fundamental limit to the number of address tags that can
be present on a primer except the size of the primer. When there are multiple address tags,
they may have the same sequence or they may have different sequences, with each different

30     sequence complementary to a different address probe. It is preferred that a primer contain
address tags that have the same sequence such that they are all complementary to a single
address probe. The address tag portion can be any length that supports specific and stable
hybridization between the address tag and the address probe. For this purpose, a length

between 10 and 35 nucleotides long is preferred, with an address tag portion 15 to 20 nucleotides long being most preferred.

## E. Fluorescent Change Probes and Primers

Fluorescent change probes and fluorescent change primers refer to all probes and primers that involve a change in fluorescence intensity or wavelength based on a change in the form or conformation of the probe or primer and nucleic acid to be detected, assayed or replicated. Examples of fluorescent change probes and primers include molecular beacons, Amplifluors, FRET probes, cleavable FRET probes, TaqMan probes, scorpion primers, fluorescent triplex oligos, fluorescent water-soluble conjugated polymers, PNA probes and QPNA probes.

Fluorescent change probes and primers can be classified according to their structure and/or function. Fluorescent change probes include hairpin quenched probes, cleavage quenched probes, cleavage activated probes, and fluorescent activated probes. Fluorescent change primers include stem quenched primers and hairpin quenched primers. The use of several types of fluorescent change probes and primers are reviewed in Schweitzer and Kingsmore, Curr. Opin. Biotech. 12:21-27 (2001). Hall et al., Proc. Natl. Acad. Sci. USA 97:8272-8277 (2000), describe the use of fluorescent change probes with Invader assays.

Hairpin quenched probes are probes that when not bound to a target sequence form a hairpin structure (and, typically, a loop) that brings a fluorescent label and a quenching moiety into proximity such that fluorescence from the label is quenched. When the probe binds to a target sequence, the stem is disrupted, the quenching moiety is no longer in proximity to the fluorescent label and fluorescence increases. Examples of hairpin quenched probes are molecular beacons, fluorescent triplex oligos, and QPNA probes.

Cleavage activated probes are probes where fluorescence is increased by cleavage of the probe. Cleavage activated probes can include a fluorescent label and a quenching moiety in proximity such that fluorescence from the label is quenched. When the probe is clipped or digested (typically by the 5'-3' exonuclease activity of a polymerase during amplification), the quenching moiety is no longer in proximity to the fluorescent label and fluorescence increases. TaqMan probes (Holland et al., Proc. Natl. Acad. Sci. USA 88:7276-7280 (1991)) are an example of cleavage activated probes.

Cleavage quenched probes are probes where fluorescence is decreased or altered by cleavage of the probe. Cleavage quenched probes can include an acceptor fluorescent label and a donor moiety such that, when the acceptor and donor are in proximity, fluorescence

resonance energy transfer from the donor to the acceptor causes the acceptor to fluoresce. The probes are thus fluorescent, for example, when hybridized to a target sequence. When the probe is clipped or digested (typically by the 5'-3' exonuclease activity of a polymerase during amplification), the donor moiety is no longer in proximity to the acceptor fluorescent

5      label and fluorescence from the acceptor decreases. If the donor moiety is itself a fluorescent label, it can release energy as fluorescence (typically at a different wavelength than the fluorescence of the acceptor) when not in proximity to an acceptor. The overall effect would then be a reduction of acceptor fluorescence and an increase in donor fluorescence. Donor fluorescence in the case of cleavage quenched probes is equivalent to

10     fluorescence generated by cleavage activated probes with the acceptor being the quenching moiety and the donor being the fluorescent label. Cleavable FRET (fluorescence resonance energy transfer) probes are an example of cleavage quenched probes.

Fluorescent activated probes are probes or pairs of probes where fluorescence is increased or altered by hybridization of the probe to a target sequence. Fluorescent

15     activated probes can include an acceptor fluorescent label and a donor moiety such that, when the acceptor and donor are in proximity (when the probes are hybridized to a target sequence), fluorescence resonance energy transfer from the donor to the acceptor causes the acceptor to fluoresce. Fluorescent activated probes are typically pairs of probes designed to hybridize to adjacent sequences such that the acceptor and donor are brought into proximity.

20     Fluorescent activated probes can also be single probes containing both a donor and acceptor where, when the probe is not hybridized to a target sequence, the donor and acceptor are not in proximity but where the donor and acceptor are brought into proximity when the probe hybridized to a target sequence. This can be accomplished, for example, by placing the donor and acceptor on opposite ends a the probe and placing target complement sequences

25     at each end of the probe where the target complement sequences are complementary to adjacent sequences in a target sequence. If the donor moiety of a fluorescent activated probe is itself a fluorescent label, it can release energy as fluorescence (typically at a different wavelength than the fluorescence of the acceptor) when not in proximity to an acceptor (that is, when the probes are not hybridized to the target sequence). When the

30     probes hybridize to a target sequence, the overall effect would then be a reduction of donor fluorescence and an increase in acceptor fluorescence. FRET probes are an example of fluorescent activated probes.

Stem quenched primers are primers that when not hybridized to a complementary sequence form a stem structure (either an intramolecular stem structure or an intermolecular stem structure) that brings a fluorescent label and a quenching moiety into proximity such that fluorescence from the label is quenched. When the primer binds to a complementary

5     sequence, the stem is disrupted, the quenching moiety is no longer in proximity to the fluorescent label and fluorescence increases. In the disclosed method, stem quenched primers are used as primers for nucleic acid synthesis and thus become incorporated into the synthesized or amplified nucleic acid. Examples of stem quenched primers are peptide nucleic acid quenched primers and hairpin quenched primers.

10     Peptide nucleic acid quenched primers are primers associated with a peptide nucleic acid quencher or a peptide nucleic acid fluor to form a stem structure. The primer contains a fluorescent label or a quenching moiety and is associated with either a peptide nucleic acid quencher or a peptide nucleic acid fluor, respectively. This puts the fluorescent label in proximity to the quenching moiety. When the primer is replicated, the peptide nucleic acid

15     is displaced, thus allowing the fluorescent label to produce a fluorescent signal.

Hairpin quenched primers are primers that when not hybridized to a complementary sequence form a hairpin structure (and, typically, a loop) that brings a fluorescent label and a quenching moiety into proximity such that fluorescence from the label is quenched. When the primer binds to a complementary sequence, the stem is disrupted, the quenching moiety

20     is no longer in proximity to the fluorescent label and fluorescence increases. Hairpin quenched primers are typically used as primers for nucleic acid synthesis and thus become incorporated into the synthesized or amplified nucleic acid. Examples of hairpin quenched primers are Amplifluor primers (Nazerenko et al., Nucleic Acids Res. 25:2516-2521 (1997)) and scorpion primers (Thelwell et al., Nucleic Acids Res. 28(19):3752-3761 (2000)).

25     Cleavage activated primers are similar to cleavage activated probes except that they are primers that are incorporated into replicated strands and are then subsequently cleaved. Little et al., Clin. Chem. 45:777-784 (1999), describe the use of cleavage activated primers.

**F. Lysis Solution**

In the disclosed method, the cells can be exposed to alkaline conditions by mixing

30     the cells with a lysis solution. A lysis solution is generally a solution that can raise the pH of a cell solution sufficiently to cause cell lysis. Denaturing solutions can be used as lysis solutions so long as the denaturing solution can have the effects required of lysis solutions. In some embodiments, the lysis solution can comprises a base, such as an aqueous base.

Useful bases include potassium hydroxide, sodium hydroxide, potassium acetate, sodium acetate, ammonium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, calcium carbonate, ammonia, aniline, benzylamine, n-butylamine, diethylamine, dimethylamine, diphenylamine, ethylamine,

5      ethylenediamine, methylamine, N-methylaniline, morpholine, pyridine, triethylamine, trimethylamine, aluminum hydroxide, rubidium hydroxide, cesium hydroxide, strontium hydroxide, barium hydroxide, and DBU (1,8-diazobicyclo[5,4,0]undec-7-ene). Useful formulations of lysis solution include lysis solution comprising 400 mM KOH, lysis solution comprising 400 mM KOH and 10 mM EDTA, lysis solution comprising 400 mM

10     KOH, 100 mM dithiothreitol, and 10 mM EDTA, and lysis solution consisting of 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA. Other useful formulations of lysis solution include lysis solution comprising 100 mM KOH, lysis solution comprising 100 mM KOH and 2.5 mM EDTA, lysis solution comprising 100 mM KOH, 25 mM dithiothreitol, and 2.5 mM EDTA, and lysis solution consisting of 100 mM KOH, 25 mM

15     dithiothreitol, and 2.5 mM EDTA. Useful lysis solutions can have a pH of 8. Lysis solutions can be diluted prior to use. In such cases, the amount of lysis solution added to a reaction generally could be increased proportionally.

In some embodiments, the lysis solution can comprise a plurality of basic agents. As used herein, a basic agent is a compound, composition or solution that results in alkaline

20     conditions. In some embodiments, the lysis solution can comprise a buffer. Useful buffers include phosphate buffers, "Good" buffers (such as BES, BICINE, CAPS, EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES, and TRICINE), sodium cacodylate, sodium citrate, triethylammonium acetate, triethylammonium bicarbonate, Tris, Bis-tris, and Bis-tris propane. The lysis solution can comprise a plurality of buffering agents. As used herein, a

25     buffering agent is a compound, composition or solution that acts as a buffer. An alkaline buffering agent is a buffering agent that results in alkaline conditions. In some embodiments, the lysis solution can comprise a combination of one or more bases, basic agents, buffers and buffering agents.

The amount of lysis solution mixed with the cells can be that amount that causes a

30     substantial number of cells to lyse or those that cause a sufficient number of cells to lyse. Generally, this volume will be a function of the pH of the cell/lysis solution mixture. Thus, the amount of lysis solution to mix with cells can be determined generally from the volume of the cells and the alkaline concentration of the lysis buffer. For example, a smaller

volume of a lysis solution with a stronger base and/or higher concentration of base would be needed to create sufficient alkaline conditions than the volume needed of a lysis solution with a weaker base and/or lower concentration of base. The lysis solution can be formulated such that the cells are mixed with an equal volume of the lysis solution (to

5    produce the desired alkaline conditions).

For example, lysis solutions can be solutions that have a pH of from about 8.0 to about 13.0, from about 8.5 to about 13.0, from about 9.0 to about 13.0, from about 9.5 to about 13.0, from about 10.0 to about 13.0, from about 10.5 to about 13.0, from about 11.0 to about 13.0, from about 11.5 to about 13.0, from about 12.0 to about 13.0, from about 8.0 to

10   about 12.0, from about 8.5 to about 12.0, from about 9.0 to about 12.0, from about 9.5 to about 12.0, from about 10.0 to about 12.0, from about 10.5 to about 12.0, from about 11.0 to about 12.0, from about 11.5 to about 12.0, from about 8.0 to about 11.5, from about 8.5 to about 11.5, from about 9.0 to about 11.5, from about 9.5 to about 11.5, from about 10.0 to about 11.5, from about 10.5 to about 11.5, from about 11.0 to about 11.5, from about 8.0 to

15   about 11.0, from about 8.5 to about 11.0, from about 9.0 to about 11.0, from about 9.5 to about 11.0, from about 10.0 to about 11.0, from about 10.5 to about 11.0, from about 8.0 to about 10.5, from about 8.5 to about 10.5, from about 9.0 to about 10.5, from about 9.5 to about 10.5, from about 10.0 to about 10.5, from about 8.0 to about 10.0, from about 8.5 to about 10.0, from about 9.0 to about 10.0, from about 9.5 to about 10.0, from about 8.0 to

20   about 9.5, from about 8.5 to about 9.5, from about 9.0 to about 9.5, about 8.0, about 8.5, about 9.0, about 9.5, about 10.0, about 10.5, about 11.0, about 11.5, about 12.0, about 12.5, or about 13.0.

Lysis solutions can have, for example, component concentrations of from about 10 mM to about 1 M, from about 10 mM to about 900 mM, from about 10 mM to about 800

25   mM, from about 10 mM to about 700 mM, from about 10 mM to about 600 mM, from about 10 mM to about 500 mM, from about 10 mM to about 400 mM, from about 10 mM to about 300 mM, from about 10 mM to about 200 mM, from about 10 mM to about 100 mM, from about 10 mM to about 90 mM, from about 10 mM to about 80 mM, from about 10 mM to about 70 mM, from about 10 mM to about 60 mM, from about 10 mM to about 50

30   mM, from about 10 mM to about 40 mM, from about 10 mM to about 30 mM, from about 10 mM to about 20 mM, from about 20 mM to about 1 M, from about 20 mM to about 900 mM, from about 20 mM to about 800 mM, from about 20 mM to about 700 mM, from about 20 mM to about 600 mM, from about 20 mM to about 500 mM, from about 20 mM to

about 400 mM, from about 20 mM to about 300 mM, from about 20 mM to about 200 mM, from about 20 mM to about 100 mM, from about 20 mM to about 90 mM, from about 20 mM to about 80 mM, from about 20 mM to about 70 mM, from about 20 mM to about 60 mM, from about 20 mM to about 50 mM, from about 20 mM to about 40 mM, from about 20 mM to about 30 mM, from about 30 mM to about 1 M, from about 30 mM to about 900 mM, from about 30 mM to about 800 mM, from about 30 mM to about 700 mM, from about 30 mM to about 600 mM, from about 30 mM to about 500 mM, from about 30 mM to about 400 mM, from about 30 mM to about 300 mM, from about 30 mM to about 200 mM, from about 30 mM to about 100 mM, from about 30 mM to about 90 mM, from about 30 mM to about 80 mM, from about 30 mM to about 70 mM, from about 30 mM to about 60 mM, from about 30 mM to about 50 mM, from about 30 mM to about 40 mM, from about 40 mM to about 1 M, from about 40 mM to about 900 mM, from about 40 mM to about 800 mM, from about 40 mM to about 700 mM, from about 40 mM to about 600 mM, from about 40 mM to about 500 mM, from about 40 mM to about 400 mM, from about 40 mM to about 300 mM, from about 40 mM to about 200 mM, from about 40 mM to about 100 mM, from about 40 mM to about 90 mM, from about 40 mM to about 80 mM, from about 40 mM to about 70 mM, from about 40 mM to about 60 mM, from about 40 mM to about 50 mM, from about 50 mM to about 1 M, from about 50 mM to about 900 mM, from about 50 mM to about 800 mM, from about 50 mM to about 700 mM, from about 50 mM to about 600 mM, from about 50 mM to about 500 mM, from about 50 mM to about 400 mM, from about 50 mM to about 300 mM, from about 50 mM to about 200 mM, from about 50 mM to about 100 mM, from about 50 mM to about 90 mM, from about 50 mM to about 80 mM, from about 50 mM to about 70 mM, from about 50 mM to about 60 mM, from about 60 mM to about 1 M, from about 60 mM to about 900 mM, from about 60 mM to about 800 mM, from about 60 mM to about 700 mM, from about 60 mM to about 600 mM, from about 60 mM to about 500 mM, from about 60 mM to about 400 mM, from about 60 mM to about 300 mM, from about 60 mM to about 200 mM, from about 60 mM to about 100 mM, from about 60 mM to about 90 mM, from about 60 mM to about 80 mM, from about 60 mM to about 70 mM, from about 70 mM to about 1 M, from about 70 mM to about 900 mM, from about 70 mM to about 800 mM, from about 70 mM to about 700 mM, from about 70 mM to about 600 mM, from about 70 mM to about 500 mM, from about 70 mM to about 400 mM, from about 70 mM to about 300 mM, from about 70 mM to about 200 mM, from about 70 mM to about 100 mM, from about 70 mM to about 90 mM, from about 70

mM to about 80 mM, from about 80 mM to about 1 M, from about 80 mM to about 900 mM, from about 80 mM to about 800 mM, from about 80 mM to about 700 mM, from about 80 mM to about 600 mM, from about 80 mM to about 500 mM, from about 80 mM to about 400 mM, from about 80 mM to about 300 mM, from about 80 mM to about 200 mM, from about 80 mM to about 100 mM, from about 80 mM to about 90 mM, from about 90 mM to about 1 M, from about 90 mM to about 900 mM, from about 90 mM to about 800 mM, from about 90 mM to about 700 mM, from about 90 mM to about 600 mM, from about 90 mM to about 500 mM, from about 90 mM to about 400 mM, from about 90 mM to about 300 mM, from about 90 mM to about 200 mM, from about 90 mM to about 100 mM, from about 100 mM to about 1 M, from about 100 mM to about 900 mM, from about 100 mM to about 800 mM, from about 100 mM to about 700 mM, from about 100 mM to about 600 mM, from about 100 mM to about 500 mM, from about 100 mM to about 400 mM, from about 100 mM to about 300 mM, from about 100 mM to about 200 mM, from about 200 mM to about 1 M, from about 200 mM to about 900 mM, from about 200 mM to about 800 mM, from about 200 mM to about 700 mM, from about 200 mM to about 600 mM, from about 200 mM to about 500 mM, from about 200 mM to about 400 mM, from about 200 mM to about 300 mM, from about 300 mM to about 1 M, from about 300 mM to about 900 mM, from about 300 mM to about 800 mM, from about 300 mM to about 700 mM, from about 300 mM to about 600 mM, from about 300 mM to about 500 mM, from about 300 mM to about 400 mM, from about 400 mM to about 1 M, from about 400 mM to about 900 mM, from about 400 mM to about 800 mM, from about 400 mM to about 700 mM, from about 400 mM to about 600 mM, from about 400 mM to about 500 mM, from about 500 mM to about 1 M, from about 500 mM to about 900 mM, from about 500 mM to about 800 mM, from about 500 mM to about 700 mM, from about 500 mM to about 600 mM, from about 600 mM to about 1 M, from about 600 mM to about 900 mM, from about 600 mM to about 800 mM, from about 600 mM to about 700 mM, from about 700 mM to about 1 M, from about 700 mM to about 900 mM, from about 700 mM to about 800 mM, from about 800 mM to about 1 M, from about 800 mM to about 900 mM, from about 900 mM to about 1 M, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, about 100 mM, about 200 mM, about 300 mM, about 400 mM, about 500 mM, about 600 mM, about 700 mM, about 800 mM, about 900 mM, or about 1 M.

Final concentrations of lysis solution components (after mixing with samples) can be, for example, from about 10 mM to about 1 M, from about 10 mM to about 900 mM, from about 10 mM to about 800 mM, from about 10 mM to about 700 mM, from about 10 mM to about 600 mM, from about 10 mM to about 500 mM, from about 10 mM to about

5   400 mM, from about 10 mM to about 300 mM, from about 10 mM to about 200 mM, from about 10 mM to about 100 mM, from about 10 mM to about 90 mM, from about 10 mM to about 80 mM, from about 10 mM to about 70 mM, from about 10 mM to about 60 mM, from about 10 mM to about 50 mM, from about 10 mM to about 40 mM, from about 10 mM to about 30 mM, from about 10 mM to about 20 mM, from about 20 mM to about 1 M,

10   from about 20 mM to about 900 mM, from about 20 mM to about 800 mM, from about 20 mM to about 700 mM, from about 20 mM to about 600 mM, from about 20 mM to about 500 mM, from about 20 mM to about 400 mM, from about 20 mM to about 300 mM, from about 20 mM to about 200 mM, from about 20 mM to about 100 mM, from about 20 mM to about 90 mM, from about 20 mM to about 80 mM, from about 20 mM to about 70 mM,

15   from about 20 mM to about 60 mM, from about 20 mM to about 50 mM, from about 20 mM to about 40 mM, from about 20 mM to about 30 mM, from about 30 mM to about 1 M, from about 30 mM to about 900 mM, from about 30 mM to about 800 mM, from about 30 mM to about 700 mM, from about 30 mM to about 600 mM, from about 30 mM to about 500 mM, from about 30 mM to about 400 mM, from about 30 mM to about 300 mM, from

20   about 30 mM to about 200 mM, from about 30 mM to about 100 mM, from about 30 mM to about 90 mM, from about 30 mM to about 80 mM, from about 30 mM to about 70 mM, from about 30 mM to about 60 mM, from about 30 mM to about 50 mM, from about 30 mM to about 40 mM, from about 40 mM to about 1 M, from about 40 mM to about 900 mM, from about 40 mM to about 800 mM, from about 40 mM to about 700 mM, from

25   about 40 mM to about 600 mM, from about 40 mM to about 500 mM, from about 40 mM to about 400 mM, from about 40 mM to about 300 mM, from about 40 mM to about 200 mM, from about 40 mM to about 100 mM, from about 40 mM to about 90 mM, from about 40 mM to about 80 mM, from about 40 mM to about 70 mM, from about 40 mM to about 60 mM, from about 40 mM to about 50 mM, from about 50 mM to about 1 M, from about 50

30   mM to about 900 mM, from about 50 mM to about 800 mM, from about 50 mM to about 700 mM, from about 50 mM to about 600 mM, from about 50 mM to about 500 mM, from about 50 mM to about 400 mM, from about 50 mM to about 300 mM, from about 50 mM to about 200 mM, from about 50 mM to about 100 mM, from about 50 mM to about 90 mM,

from about 50 mM to about 80 mM, from about 50 mM to about 70 mM, from about 50 mM to about 60 mM, from about 60 mM to about 1 M, from about 60 mM to about 900 mM, from about 60 mM to about 800 mM, from about 60 mM to about 700 mM, from about 60 mM to about 600 mM, from about 60 mM to about 500 mM, from about 60 mM to about 400 mM, from about 60 mM to about 300 mM, from about 60 mM to about 200 mM, from about 60 mM to about 100 mM, from about 60 mM to about 90 mM, from about 60 mM to about 80 mM, from about 60 mM to about 70 mM, from about 70 mM to about 1 M, from about 70 mM to about 900 mM, from about 70 mM to about 800 mM, from about 70 mM to about 700 mM, from about 70 mM to about 600 mM, from about 70 mM to about 500 mM, from about 70 mM to about 400 mM, from about 70 mM to about 300 mM, from about 70 mM to about 200 mM, from about 70 mM to about 100 mM, from about 70 mM to about 90 mM, from about 70 mM to about 80 mM, from about 80 mM to about 1 M, from about 80 mM to about 900 mM, from about 80 mM to about 800 mM, from about 80 mM to about 700 mM, from about 80 mM to about 600 mM, from about 80 mM to about 500 mM, from about 80 mM to about 400 mM, from about 80 mM to about 300 mM, from about 80 mM to about 200 mM, from about 80 mM to about 100 mM, from about 80 mM to about 90 mM, from about 90 mM to about 1 M, from about 90 mM to about 900 mM, from about 90 mM to about 800 mM, from about 90 mM to about 700 mM, from about 90 mM to about 600 mM, from about 90 mM to about 500 mM, from about 90 mM to about 400 mM, from about 90 mM to about 300 mM, from about 90 mM to about 200 mM, from about 90 mM to about 100 mM, from about 100 mM to about 1 M, from about 100 mM to about 900 mM, from about 100 mM to about 800 mM, from about 100 mM to about 700 mM, from about 100 mM to about 600 mM, from about 100 mM to about 500 mM, from about 100 mM to about 400 mM, from about 100 mM to about 300 mM, from about 100 mM to about 200 mM, from about 200 mM to about 1 M, from about 200 mM to about 900 mM, from about 200 mM to about 800 mM, from about 200 mM to about 700 mM, from about 200 mM to about 600 mM, from about 200 mM to about 500 mM, from about 200 mM to about 400 mM, from about 200 mM to about 300 mM, from about 300 mM to about 1 M, from about 300 mM to about 900 mM, from about 300 mM to about 800 mM, from about 300 mM to about 700 mM, from about 300 mM to about 600 mM, from about 300 mM to about 500 mM, from about 300 mM to about 400 mM, from about 400 mM to about 1 M, from about 400 mM to about 900 mM, from about 400 mM to about 800 mM, from about 400 mM to about 700 mM, from about 400 mM to about 600 mM, from about 400 mM to about 500

mM, from about 500 mM to about 1 M, from about 500 mM to about 900 mM, from about
500 mM to about 800 mM, from about 500 mM to about 700 mM, from about 500 mM to
about 600 mM, from about 600 mM to about 1 M, from about 600 mM to about 900 mM,
from about 600 mM to about 800 mM, from about 600 mM to about 700 mM, from about
700 mM to about 1 M, from about 700 mM to about 900 mM, from about 700 mM to about
800 mM, from about 800 mM to about 1 M, from about 800 mM to about 900 mM, from
about 900 mM to about 1 M, about 10 mM, about 20 mM, about 30 mM, about 40 mM,
about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, about 100 mM,
about 200 mM, about 300 mM, about 400 mM, about 500 mM, about 600 mM, about 700
mM, about 800 mM, about 900 mM, or about 1 M.

The lysis solution can be composed of multiple solutions and/or components that
can be added to cells separately or combined in different combinations prior to addition to
cells. Thus, for example, a solution of 400 mM KOH and 10 mM EDTA and a solution of
100 mM dithiothreitol can be added to the cells separately. Similarly, the disclosed kits can
be composed of multiple solutions and/or components to be combined to form a lysis
solution prior to addition to cells or for separate addition to cells. Stock lysis solutions can
be diluted to form final lysis solutions for use in the disclosed method. Stock lysis solutions
can have any concentration described herein for lysis solutions or any concentration that is
more concentrated than any lysis solution or lysis solution concentration described herein.
The final concentration of lysis solution components (after mixing with samples) can be any
concentration described herein for lysis solutions. Useful final concentrations of lysis
solution components can be 50 mM KOH, 12.5 mM dithiothreitol, and 1.25 mM EDTA.

**G. Stabilization Solution**

In the disclosed method, the pH of the cell lysate or sample can be reduced to form a
stabilized or neutralized cell lysate or stabilized or neutralized sample. A stabilization
solution is generally a solution that can reduce the pH of a cell lysate or sample exposed to
alkaline conditions as described elsewhere herein. In some embodiments, the stabilization
solution can comprise an acid. Useful acids include hydrochloric acid, sulfuric acid,
phosphoric acid, acetic acid, acetylsalicylic acid, ascorbic acid, carbonic acid, citric acid,
formic acid, nitric acid, perchloric acid, HF, HBr, HI, $H_2S$, HCN, HSCN, HClO,
monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, and any carboxylic acid
(ethanoic, propanoic, butanoic, etc., including both linear or branched chain carboxylic
acids). In some embodiments, the stabilization solution can comprise a buffer. Useful

buffers include Tris-HCl, HEPES, "Good" buffers (such as BES, BICINE, CAPS, EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES, and TRICINE), sodium cacodylate, sodium citrate, triethylammonium acetate, triethylammonium bicarbonate, Tris, Bis-tris, and Bis-tris propane. Useful formulations of stabilization solutions include stabilization solution

5    comprising 800 mM Tris-HCl; stabilization solution comprising 800 mM Tris-HCl at pH 4.1, and stabilization solution consisting of 800 mM Tris-HCl, pH 4.1. Useful formulations of stabilization solutions include stabilization solution comprising 800 mM Tris-HCl at pH 4, and stabilization solution consisting of 800 mM Tris-HCl, pH 4. Other useful formulations of stabilization solutions include stabilization solution comprising 160 mM

10   Tris-HCl; stabilization solution comprising 160 mM Tris-HCl at pH 4.1, and stabilization solution consisting of 160 mM Tris-HCl, pH 4.1. Other useful formulations of stabilization solutions include stabilization solution comprising 160 mM Tris-HCl; stabilization solution comprising 160 mM Tris-HCl at pH 4, and stabilization solution consisting of 160 mM Tris-HCl, pH 4. Stabilization solutions can be diluted prior to use. In such cases, the

15   amount of stabilization solution added to a reaction generally could be increased proportionally.

    In some embodiments, the stabilization solution can comprise a plurality of acidic agents. As used herein, an acidic agent is a compound, composition or solution that forms an acid in solution. In some embodiments, the stabilization solution can comprise a

20   plurality of buffering agents. An acidic buffering agent is a buffering agent that forms an acid in solution. In some embodiments, the stabilization solution can comprise a combination of one or more acids, acidic agents, buffers and buffering agents.

    A stabilized cell lysate or stabilized samples is a cell lysate or sample the pH of which is in the neutral range (from about pH 6.0 to about pH 9.0). Useful stabilized cell

25   lysates and samples have a pH that allows replication of nucleic acids in the cell lysate. For example, the pH of the stabilized cell lysate or sample is usefully at a pH at which the DNA polymerase can function. The pH of the cell lysate or sample can be reduced by mixing the cell lysate or sample with a stabilization solution.

    The amount of stabilization solution mixed with the cell lysate or sample can be that

30   amount that causes a reduction in pH to the neutral range (or other desired pH value). Generally, this volume will be a function of the pH of the cell lysate/stabilization solution mixture or of the sample/stabilization solution mixture. Thus, the amount of stabilization solution to mix with the cell lysate or sample can be determined generally from the volume

of the cell lysate or sample, its pH and buffering capacity, and the acidic concentration of the stabilization buffer. For example, a smaller volume of a stabilization solution with a stronger acid and/or higher concentration of acid would be needed to reduce the pH sufficiently than the volume needed of a stabilization solution with a weaker acid and/or

5        lower concentration of acid. The stabilization solution can be formulated such that the cell lysate or sample is mixed with an equal volume of the stabilization solution (to produce the desired pH).

For example, stabilization solutions can be solutions that have a pH of from about 1.0 to about 6.0, from about 2.0 to about 6.0, from about 3.0 to about 6.0, from about 3.5 to

10       about 6.0, from about 4.0 to about 6.0, from about 4.5 to about 6.0, from about 5.0 to about 6.0, from about 5.5 to about 6.0, from about 1.0 to about 5.5, from about 2.0 to about 5.5, from about 3.0 to about 5.5, from about 3.5 to about 5.5, from about 4.0 to about 5.5, from about 4.5 to about 5.5, from about 5.0 to about 5.5, from about 1.0 to about 5.0, from about 2.0 to about 5.0, from about 3.0 to about 5.0, from about 3.5 to about 5.0, from about 4.0 to

15       about 5.0, from about 4.5 to about 5.0, from about 1.0 to about 4.5, from about 2.0 to about 4.5, from about 3.0 to about 4.5, from about 3.5 to about 4.5, from about 4.0 to about 4.5, from about 1.0 to about 4.0, from about 2.0 to about 4.0, from about 3.0 to about 4.0, from about 3.5 to about 4.0, from about 1.0 to about 3.5, from about 2.0 to about 3.5, from about 3.0 to about 3.5, from about 1.0 to about 3.0, from about 2.0 to about 3.0, from about 1.0 to

20       about 2.5, from about 2.0 to about 2.5, from about 1.0 to about 2.0, about 1.0, about 2.0, about 2.5, about 3.0, about 3.5, about 4.0, about 4.5, about 5.0, about 5.5, or about 6.0.

Stabilization solutions can have, for example, component concentrations of from about 100 mM to about 1 M, from about 100 mM to about 900 mM, from about 100 mM to about 800 mM, from about 100 mM to about 700 mM, from about 100 mM to about 600

25       mM, from about 100 mM to about 500 mM, from about 100 mM to about 400 mM, from about 100 mM to about 300 mM, from about 100 mM to about 200 mM, from about 200 mM to about 1 M, from about 200 mM to about 900 mM, from about 200 mM to about 800 mM, from about 200 mM to about 700 mM, from about 200 mM to about 600 mM, from about 200 mM to about 500 mM, from about 200 mM to about 400 mM, from about 200

30       mM to about 300 mM, from about 300 mM to about 1 M, from about 300 mM to about 900 mM, from about 300 mM to about 800 mM, from about 300 mM to about 700 mM, from about 300 mM to about 600 mM, from about 300 mM to about 500 mM, from about 300 mM to about 400 mM, from about 400 mM to about 1 M, from about 400 mM to about 900

mM, from about 400 mM to about 800 mM, from about 400 mM to about 700 mM, from about 400 mM to about 600 mM, from about 400 mM to about 500 mM, from about 500 mM to about 1 M, from about 500 mM to about 900 mM, from about 500 mM to about 800 mM, from about 500 mM to about 700 mM, from about 500 mM to about 600 mM, from about 600 mM to about 1 M, from about 600 mM to about 900 mM, from about 600 mM to about 800 mM, from about 600 mM to about 700 mM, from about 700 mM to about 1 M, from about 700 mM to about 900 mM, from about 700 mM to about 800 mM, from about 800 mM to about 1 M, from about 800 mM to about 900 mM, from about 900 mM to about 1 M, about 100 mM, about 200 mM, about 300 mM, about 400 mM, about 500 mM, about 600 mM, about 700 mM, about 800 mM, about 900 mM, or about 1 M.

Final concentrations of stabilization solution components can be, for example, from about 100 mM to about 1 M, from about 100 mM to about 900 mM, from about 100 mM to about 800 mM, from about 100 mM to about 700 mM, from about 100 mM to about 600 mM, from about 100 mM to about 500 mM, from about 100 mM to about 400 mM, from about 100 mM to about 300 mM, from about 100 mM to about 200 mM, from about 200 mM to about 1 M, from about 200 mM to about 900 mM, from about 200 mM to about 800 mM, from about 200 mM to about 700 mM, from about 200 mM to about 600 mM, from about 200 mM to about 500 mM, from about 200 mM to about 400 mM, from about 200 mM to about 300 mM, from about 300 mM to about 1 M, from about 300 mM to about 900 mM, from about 300 mM to about 800 mM, from about 300 mM to about 700 mM, from about 300 mM to about 600 mM, from about 300 mM to about 500 mM, from about 300 mM to about 400 mM, from about 400 mM to about 1 M, from about 400 mM to about 900 mM, from about 400 mM to about 800 mM, from about 400 mM to about 700 mM, from about 400 mM to about 600 mM, from about 400 mM to about 500 mM, from about 500 mM to about 1 M, from about 500 mM to about 900 mM, from about 500 mM to about 800 mM, from about 500 mM to about 700 mM, from about 500 mM to about 600 mM, from about 600 mM to about 1 M, from about 600 mM to about 900 mM, from about 600 mM to about 800 mM, from about 600 mM to about 700 mM, from about 700 mM to about 1 M, from about 700 mM to about 900 mM, from about 700 mM to about 800 mM, from about 800 mM to about 1 M, from about 800 mM to about 900 mM, from about 900 mM to about 1 M, about 100 mM, about 200 mM, about 300 mM, about 400 mM, about 500 mM, about 600 mM, about 700 mM, about 800 mM, about 900 mM, or about 1 M.

The stabilization solution can be composed of multiple solutions and/or components that can be added to cell lysates and samples separately or combined in different combinations prior to addition to cell lysates and samples. Thus, for example, a solution of a buffer and a solution of an acid can be added to the cells separately. Similarly, the

5   disclosed kits can be composed of multiple solutions and/or components to be combined to form a stabilization solution prior to addition to cell lysates or samples or for separate addition to cell lysates or samples. Stock stablization solutions can be diluted to form final stabilization solutions for use in the disclosed method. Stock stabilization solutions can have any concentration described herein for stabilization solutions or any concentration that

10   is more concentrated than any stabilization solution or stabilization solution concentration described herein. The final concentration of stabilization solution components (after mixing with samples) can be any concentration described herein for stabilization solutions. Useful final concentrations of lysis solution components can be 80 mM Tris-HCl.

As used herein, a neutralization solution is a form of stabilization solution.

15   Reference to neutralized cell lysates, neutralized sample, and other neutralized components or solutions is considered the equivalent of a stabilized cell lysate, stabilized sample, or other stabilized component or solution.

**H. Denaturing Solution**

In some forms of the disclosed method, the DNA samples can be exposed to

20   denaturing conditions by mixing the sample with a denaturing solution. A denaturing solution is generally a solution that can raise the pH of a sample sufficiently to cause, in combination with other conditions such as heating, substantial denaturation of DNA in the DNA sample. Substantial denaturation refers to denaturation of 90% or more of the nucleotides in 90% or more of the DNA molecules in a sample. In this context,

25   denaturation of nucleotides refers to unpaired nucleotides whether physically denatured by treatment or already unpaired in the sample. Lysis solutions can be used as denaturing solutions so long as the lysis solution has the effects required of denaturing solutions.

In some embodiments, the denaturing solution can comprises a base, such as an aqueous base. Useful bases include potassium hydroxide, sodium hydroxide, potassium

30   acetate, sodium acetate, ammonium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, calcium carbonate, ammonia, aniline, benzylamine, n-butylamine, diethylamine, dimethylamine, diphenylamine, ethylamine, ethylenediamine, methylamine, N-methylaniline, morpholine,

71

pyridine, triethylamine, trimethylamine, aluminum hydroxide, rubidium hydroxide, cesium hydroxide, strontium hydroxide, barium hydroxide, and DBU (1,8-diazobicyclo[5,4,0]undec-7-ene). Useful formulations of denaturing solution include denaturing solution comprising about 150 mM to about 500 mM NaOH, denaturing solution comprising about 150 mM to about 500 mM NaOH, and denaturing solution consisting of about 150 mM to about 500 mM NaOH. Denaturing solutions can be diluted prior to use. In such cases, the amount of denaturing solution added to a reaction generally could be increased proportionally.

In some embodiments, the denaturing solution can comprise a plurality of basic agents. As used herein, a basic agent is a compound, composition or solution that results in denaturing conditions. In some embodiments, the denaturing solution can comprise a buffer. Useful buffers include phosphate buffers, "Good" buffers (such as BES, BICINE, CAPS, EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES, and TRICINE), sodium cacodylate, sodium citrate, triethylammonium acetate, triethylammonium bicarbonate, Tris, Bis-tris, and Bis-tris propane. The denaturing solution can comprise a plurality of buffering agents. As used herein, a buffering agent is a compound, composition or solution that acts as a buffer. An alkaline buffering agent is a buffering agent that results in alkaline conditions. In some embodiments, the denaturing solution can comprise a combination of one or more bases, basic agents, buffers and buffering agents.

The amount of denaturing solution mixed with the DNA samples can be that amount that causes, in combination with other conditions such as heating, substantial denaturation of DNA in the DNA sample. Generally, this volume will be a function of the pH, ionic strength, and temperature of the sample/denaturing solution mixture. Thus, the amount of denaturing solution to mix with DNA samples can be determined generally from the volume of the DNA sample, the alkaline concentration of the denaturing buffer, and the temperature to which the resulting mixture will be heated. For example, at a given temperature, a smaller volume of a denaturing solution with a stronger base and/or higher concentration of base would be needed to create sufficient denaturing conditions than the volume needed of a denaturing solution with a weaker base and/or lower concentration of base. The denaturing solution can be formulated such that the DNA samples are mixed with, for example, one tenth volume of the denaturing solution (to produce the desired denaturing conditions).

For example, denaturing solutions can be solutions that have a pH of from about 9.0 to about 13.0, from about 9.5 to about 13.0, from about 10.0 to about 13.0, from about 10.5

to about 13.0, from about 11.0 to about 13.0, from about 11.5 to about 13.0, from about 12.0 to about 13.0, from about 9.0 to about 12.0, from about 9.5 to about 12.0, from about 10.0 to about 12.0, from about 10.5 to about 12.0, from about 11.0 to about 12.0, from about 11.5 to about 12.0, from about 9.0 to about 11.5, from about 9.5 to about 11.5, from about 10.0 to about 11.5, from about 10.5 to about 11.5, from about 11.0 to about 11.5, from about 9.0 to about 11.0, from about 9.5 to about 11.0, from about 10.0 to about 11.0, from about 10.5 to about 11.0, from about 9.0 to about 10.5, from about 9.5 to about 10.5, from about 10.0 to about 10.5, from about 9.0 to about 10.0, from about 9.5 to about 10.0, from about 9.0 to about 9.5, about 9.0, about 9.5, about 10.0, about 10.5, about 11.0, about 11.5, about 12.0, about 12.5, or about 13.0.

Denaturing solutions can have, for example, component concentrations of from about 10 mM to about 1 M, from about 10 mM to about 900 mM, from about 10 mM to about 800 mM, from about 10 mM to about 700 mM, from about 10 mM to about 600 mM, from about 10 mM to about 500 mM, from about 10 mM to about 400 mM, from about 10 mM to about 300 mM, from about 10 mM to about 200 mM, from about 10 mM to about 100 mM, from about 10 mM to about 90 mM, from about 10 mM to about 80 mM, from about 10 mM to about 70 mM, from about 10 mM to about 60 mM, from about 10 mM to about 50 mM, from about 10 mM to about 40 mM, from about 10 mM to about 30 mM, from about 10 mM to about 20 mM, from about 20 mM to about 1 M, from about 20 mM to about 900 mM, from about 20 mM to about 800 mM, from about 20 mM to about 700 mM, from about 20 mM to about 600 mM, from about 20 mM to about 500 mM, from about 20 mM to about 400 mM, from about 20 mM to about 300 mM, from about 20 mM to about 200 mM, from about 20 mM to about 100 mM, from about 20 mM to about 90 mM, from about 20 mM to about 80 mM, from about 20 mM to about 70 mM, from about 20 mM to about 60 mM, from about 20 mM to about 50 mM, from about 20 mM to about 40 mM, from about 20 mM to about 30 mM, from about 30 mM to about 1 M, from about 30 mM to about 900 mM, from about 30 mM to about 800 mM, from about 30 mM to about 700 mM, from about 30 mM to about 600 mM, from about 30 mM to about 500 mM, from about 30 mM to about 400 mM, from about 30 mM to about 300 mM, from about 30 mM to about 200 mM, from about 30 mM to about 100 mM, from about 30 mM to about 90 mM, from about 30 mM to about 80 mM, from about 30 mM to about 70 mM, from about 30 mM to about 60 mM, from about 30 mM to about 50 mM, from about 30 mM to about 40 mM, from about 40 mM to about 1 M, from about 40 mM to about 900 mM, from about 40 mM

73

to about 800 mM, from about 40 mM to about 700 mM, from about 40 mM to about 600 mM, from about 40 mM to about 500 mM, from about 40 mM to about 400 mM, from about 40 mM to about 300 mM, from about 40 mM to about 200 mM, from about 40 mM to about 100 mM, from about 40 mM to about 90 mM, from about 40 mM to about 80 mM,

from about 40 mM to about 70 mM, from about 40 mM to about 60 mM, from about 40 mM to about 50 mM, from about 50 mM to about 1 M, from about 50 mM to about 900 mM, from about 50 mM to about 800 mM, from about 50 mM to about 700 mM, from about 50 mM to about 600 mM, from about 50 mM to about 500 mM, from about 50 mM to about 400 mM, from about 50 mM to about 300 mM, from about 50 mM to about 200 mM,

from about 50 mM to about 100 mM, from about 50 mM to about 90 mM, from about 50 mM to about 80 mM, from about 50 mM to about 70 mM, from about 50 mM to about 60 mM, from about 60 mM to about 1 M, from about 60 mM to about 900 mM, from about 60 mM to about 800 mM, from about 60 mM to about 700 mM, from about 60 mM to about 600 mM, from about 60 mM to about 500 mM, from about 60 mM to about 400 mM, from about 60 mM to about 300 mM, from about 60 mM to about 200 mM, from about 60 mM to about 100 mM, from about 60 mM to about 90 mM, from about 60 mM to about 80 mM,

from about 60 mM to about 70 mM, from about 70 mM to about 1 M, from about 70 mM to about 900 mM, from about 70 mM to about 800 mM, from about 70 mM to about 700 mM, from about 70 mM to about 600 mM, from about 70 mM to about 500 mM, from about 70 mM to about 400 mM, from about 70 mM to about 300 mM, from about 70 mM to about 200 mM, from about 70 mM to about 100 mM, from about 70 mM to about 90 mM, from about 70 mM to about 80 mM, from about 80 mM to about 1 M, from about 80 mM to about 900 mM, from about 80 mM to about 800 mM, from about 80 mM to about 700 mM, from about 80 mM to about 600 mM, from about 80 mM to about 500 mM, from about 80 mM to about 400 mM, from about 80 mM to about 300 mM, from about 80 mM to about 200 mM, from about 80 mM to about 100 mM, from about 80 mM to about 90 mM, from about 90 mM to about 1 M, from about 90 mM to about 900 mM, from about 90 mM to about 800 mM, from about 90 mM to about 700 mM, from about 90 mM to about 600 mM, from about 90 mM to about 500 mM, from about 90 mM to about 400 mM, from about 90 mM to about 300 mM, from about 90 mM to about 200 mM, from about 90 mM to about 100 mM, from about 100 mM to about 1 M, from about 100 mM to about 900 mM, from about 100 mM to about 800 mM, from about 100 mM to about 700 mM, from about 100 mM to about 600 mM, from about 100 mM to about 500 mM, from about 100 mM to about

400 mM, from about 100 mM to about 300 mM, from about 100 mM to about 200 mM, from about 200 mM to about 1 M, from about 200 mM to about 900 mM, from about 200 mM to about 800 mM, from about 200 mM to about 700 mM, from about 200 mM to about 600 mM, from about 200 mM to about 500 mM, from about 200 mM to about 400 mM,

5       from about 200 mM to about 300 mM, from about 300 mM to about 1 M, from about 300 mM to about 900 mM, from about 300 mM to about 800 mM, from about 300 mM to about 700 mM, from about 300 mM to about 600 mM, from about 300 mM to about 500 mM, from about 300 mM to about 400 mM, from about 400 mM to about 1 M, from about 400 mM to about 900 mM, from about 400 mM to about 800 mM, from about 400 mM to about

10      700 mM, from about 400 mM to about 600 mM, from about 400 mM to about 500 mM, from about 500 mM to about 1 M, from about 500 mM to about 900 mM, from about 500 mM to about 800 mM, from about 500 mM to about 700 mM, from about 500 mM to about 600 mM, from about 600 mM to about 1 M, from about 600 mM to about 900 mM, from about 600 mM to about 800 mM, from about 600 mM to about 700 mM, from about 700

15      mM to about 1 M, from about 700 mM to about 900 mM, from about 700 mM to about 800 mM, from about 800 mM to about 1 M, from about 800 mM to about 900 mM, from about 900 mM to about 1 M, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, about 100 mM, about 200 mM, about 300 mM, about 400 mM, about 500 mM, about 600 mM, about 700 mM, about

20      800 mM, about 900 mM, or about 1 M.

        The denaturing solution can be composed of multiple solutions and/or components that can be added to DNA samples separately or combined in different combinations prior to addition to DNA samples. Thus, for example, a solution of a buffer and a solution of a base can be added to the samples separately. Similarly, the disclosed kits can be composed

25      of multiple solutions and/or components to be combined to form a denaturing solution prior to addition to DNA samples or for separate addition to samples. Stock denaturing solutions can be diluted to form final denaturing solutions for use in the disclosed method. Stock denaturing solutions can have any concentration described herein for denaturing solutions or any concentration that is more concentrated than any denaturing solution or denaturing

30      solution concentration described herein. The final concentration of denaturing solution components (after mixing with samples) can be any concentration described herein for denaturing solutions.

## I.  Nucleic Acid Fingerprints

The disclosed method can be used to produce replicated strands that serve as a nucleic acid fingerprint of a complex sample of nucleic acid.  Such a nucleic acid fingerprint can be compared with other, similarly prepared nucleic acid fingerprints of other

5      nucleic acid samples to allow convenient detection of differences between the samples.  The nucleic acid fingerprints can be used both for detection of related nucleic acid samples and comparison of nucleic acid samples.  For example, the presence or identity of specific organisms can be detected by producing a nucleic acid fingerprint of the test organism and comparing the resulting nucleic acid fingerprint with reference nucleic acid fingerprints

10     prepared from known organisms.  Changes and differences in gene expression patterns can also be detected by preparing nucleic acid fingerprints of mRNA from different cell samples and comparing the nucleic acid fingerprints.  The replicated strands can also be used to produce a set of probes or primers that is specific for the source of a nucleic acid sample.  The replicated strands can also be used as a library of nucleic acid sequences present in a

15     sample.  Nucleic acid fingerprints can be made up of, or derived from, whole genome amplification of a sample such that the entire relevant nucleic acid content of the sample is substantially represented, or from multiple strand displacement amplification of selected target sequences within a sample.

Nucleic acid fingerprints can be stored or archived for later use.  For example,

20     replicated strands produced in the disclosed method can be physically stored, either in solution, frozen, or attached or adhered to a solid-state substrate such as an array.  Storage in an array is useful for providing an archived probe set derived from the nucleic acids in any sample of interest.  As another example, informational content of, or derived from, nucleic acid fingerprints can also be stored.  Such information can be stored, for example, in

25     or as computer readable media.  Examples of informational content of nucleic acid fingerprints include nucleic acid sequence information (complete or partial); differential nucleic acid sequence information such as sequences present in one sample but not another; hybridization patterns of replicated strands to, for example, nucleic acid arrays, sets, chips, or other replicated strands.  Numerous other data that is or can be derived from nucleic acid

30     fingerprints and replicated strands produced in the disclosed method can also be collected, used, saved, stored, and/or archived.

Nucleic acid fingerprints can also contain or be made up of other information derived from the information generated in the disclosed method, and can be combined with

information obtained or generated from any other source. The informational nature of nucleic acid fingerprints produced using the disclosed method lends itself to combination and/or analysis using known bioinformatics systems and methods.

Nucleic acid fingerprints of nucleic acid samples can be compared to a similar nucleic acid fingerprint derived from any other sample to detect similarities and differences in the samples (which is indicative of similarities and differences in the nucleic acids in the samples). For example, a nucleic acid fingerprint of a first nucleic acid sample can be compared to a nucleic acid fingerprint of a sample from the same type of organism as the first nucleic acid sample, a sample from the same type of tissue as the first nucleic acid sample, a sample from the same organism as the first nucleic acid sample, a sample obtained from the same source but at time different from that of the first nucleic acid sample, a sample from an organism different from that of the first nucleic acid sample, a sample from a type of tissue different from that of the first nucleic acid sample, a sample from a strain of organism different from that of the first nucleic acid sample, a sample from a species of organism different from that of the first nucleic acid sample, or a sample from a type of organism different from that of the first nucleic acid sample.

The same type of tissue is tissue of the same type such as liver tissue, muscle tissue, or skin (which may be from the same or a different organism or type of organism). The same organism refers to the same individual, animal, or cell. For example, two samples taken from a patient are from the same organism. The same source is similar but broader, referring to samples from, for example, the same organism, the same tissue from the same organism, the same DNA molecule, or the same DNA library. Samples from the same source that are to be compared can be collected at different times (thus allowing for potential changes over time to be detected). This is especially useful when the effect of a treatment or change in condition is to be assessed. Samples from the same source that have undergone different treatments can also be collected and compared using the disclosed method. A different organism refers to a different individual organism, such as a different patient, a different individual animal. Different organism includes a different organism of the same type or organisms of different types. A different type of organism refers to organisms of different types such as a dog and cat, a human and a mouse, or *E. coli* and *Salmonella*. A different type of tissue refers to tissues of different types such as liver and kidney, or skin and brain. A different strain or species of organism refers to organisms differing in their species or strain designation as those terms are understood in the art.

## J.  Solid-State Detectors

Solid-state detectors are solid-state substrates or supports to which address probes or detection molecules have been coupled.  A preferred form of solid-state detector is an array detector.  An array detector is a solid-state detector to which multiple different address probes or detection molecules have been coupled in an array, grid, or other organized pattern.

Solid-state substrates for use in solid-state detectors can include any solid material to which oligonucleotides can be coupled.  This includes materials such as acrylamide, cellulose, nitrocellulose, glass, gold, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, glass, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, functionalized silane, polypropylfumerate, collagen, glycosaminoglycans, and polyamino acids.  Solid-state substrates can have any useful form including tubes, test tubes, eppendorf tubes, vessels, micro vessels, plates, wells, wells of micro well plates, wells of microtitre plates, chambers, micro fluidics chambers, micro machined chambers, sealed chambers, holes, depressions, dimples, dishes, surfaces, membranes, microarrays, fibers, glass fibers, optical fibers, woven fibers, films, beads, bottles, chips, compact disks, shaped polymers, particles and microparticles.  A chip is a rectangular or square small piece of material.  Surfaces and other reaction chambers can be sealable.  Preferred forms for solid-state substrates are thin films, beads, or chips.

Address probes immobilized on a solid-state substrate allow capture of the products of the disclosed amplification method on a solid-state detector.  Such capture provides a convenient means of washing away reaction components that might interfere with subsequent detection steps.  By attaching different address probes to different regions of a solid-state detector, different amplification products can be captured at different, and therefore diagnostic, locations on the solid-state detector.  For example, in a multiplex assay, address probes specific for numerous different amplified nucleic acids (each representing a different target sequence amplified via a different set of primers) can be immobilized in an array, each in a different location.  Capture and detection will occur only at those array locations corresponding to amplified nucleic acids for which the corresponding target sequences were present in a sample.

Methods for immobilization of oligonucleotides to solid-state substrates are well established.  Oligonucleotides, including address probes and detection probes, can be

coupled to substrates using established coupling methods. For example, suitable attachment methods are described by Pease *et al.*, *Proc. Natl. Acad. Sci. USA* **91**(11):5022-5026 (1994), and Khrapko *et al.*, *Mol Biol (Mosk) (USSR)* 25:718-730 (1991). A method for immobilization of 3'-amine oligonucleotides on casein-coated slides is described by

5       Stimpson *et al.*, *Proc. Natl. Acad. Sci. USA* **92**:6379-6383 (1995). A preferred method of attaching oligonucleotides to solid-state substrates is described by Guo *et al.*, *Nucleic Acids Res.* 22:5456-5465 (1994). Examples of nucleic acid chips and arrays, including methods of making and using such chips and arrays, are described in U.S. Patent No. 6,287,768, U.S. Patent No. 6,288,220, U.S. Patent No. 6,287,776, U.S. Patent No. 6,297,006, and U.S.

10      Patent No. 6,291,193.

### K. Solid-State Samples

        Solid-state samples are solid-state substrates or supports to which target sequences or MDA products (that is, replicated strands) have been coupled or adhered. Target sequences are preferably delivered in a target sample or assay sample. A preferred form of

15      solid-state sample is an array sample. An array sample is a solid-state sample to which multiple different target sequences have been coupled or adhered in an array, grid, or other organized pattern.

        Solid-state substrates for use in solid-state samples can include any solid material to which target sequences can be coupled or adhered. This includes materials such as

20      acrylamide, cellulose, nitrocellulose, glass, gold, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, glass, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, functionalized silane, polypropylfumerate, collagen, glycosaminoglycans, and polyamino acids. Solid-state substrates can have any useful form

25      including tubes, test tubes, eppendorf tubes, vessels, micro vessels, plates, wells, wells of micro well plates, wells of microtitre plates, chambers, micro fluidics chambers, micro machined chambers, sealed chambers, holes, depressions, dimples, dishes, surfaces, membranes, microarrays, fibers, glass fibers, optical fibers, woven fibers, films, beads, bottles, chips, compact disks, shaped polymers, particles and microparticles. A chip is a

30      rectangular or square small piece of material. Surfaces and other reaction chambers can be sealable. Preferred forms for solid-state substrates are thin films, beads, or chips.

        Target sequences immobilized on a solid-state substrate allow formation of target-specific amplified nucleic acid localized on the solid-state substrate. Such localization

79

provides a convenient means of washing away reaction components that might interfere with subsequent detection steps, and a convenient way of assaying multiple different samples simultaneously. Amplified nucleic acid can be independently formed at each site where a different sample is adhered. For immobilization of target sequences or other

5      oligonucleotide molecules to form a solid-state sample, the methods described above can be used. Nucleic acids produced in the disclosed method can be coupled or adhered to a solid-state substrate in any suitable way. For example, nucleic acids generated by multiple strand displacement can be attached by adding modified nucleotides to the 3' ends of nucleic acids produced by strand displacement replication using terminal deoxynucleotidyl transferase,

10     and reacting the modified nucleotides with a solid-state substrate or support thereby attaching the nucleic acids to the solid-state substrate or support.

A preferred form of solid-state substrate is a glass slide to which up to 256 separate target samples have been adhered as an array of small dots. Each dot is preferably from 0.1 to 2.5 mm in diameter, and most preferably around 2.5 mm in diameter. Such microarrays

15     can be fabricated, for example, using the method described by Schena *et al.*, *Science* 270:487-470 (1995). Briefly, microarrays can be fabricated on poly-L-lysine-coated microscope slides (Sigma) with an arraying machine fitted with one printing tip. The tip is loaded with 1μl of a DNA sample (0.5 mg/ml) from, for example, 96-well microtiter plates and deposited ~0.005 μl per slide on multiple slides at the desired spacing. The printed

20     slides can then be rehydrated for 2 hours in a humid chamber, snap-dried at 100°C for 1 minute, rinsed in 0.1% SDS, and treated with 0.05% succinic anhydride prepared in buffer consisting of 50% 1-methyl-2-pyrrolidinone and 50% boric acid. The DNA on the slides can then be denatured in, for example, distilled water for 2 minutes at 90°C immediately before use. Microarray solid-state samples can scanned with, for example, a laser

25     fluorescent scanner with a computer-controlled XY stage and a microscope objective. A mixed gas, multiline laser allows sequential excitation of multiple fluorophores.

**L. Detection Labels**

To aid in detection and quantitation of nucleic acids amplified using the disclosed method, detection labels can be directly incorporated into amplified nucleic acids or can be

30     coupled to detection molecules. As used herein, a detection label is any molecule that can be associated with amplified nucleic acid, directly or indirectly, and which results in a measurable, detectable signal, either directly or indirectly. Many such labels for incorporation into nucleic acids or coupling to nucleic acid probes are known to those of

skill in the art. Examples of detection labels suitable for use in the disclosed method are radioactive isotopes, fluorescent molecules, phosphorescent molecules, enzymes, antibodies, and ligands.

Examples of suitable fluorescent labels include fluorescein isothiocyanate (FITC),
5    5,6-carboxymethyl fluorescein, Texas red, nitrobenz-2-oxa-1,3-diazol-4-yl (NBD), coumarin, dansyl chloride, rhodamine, amino-methyl coumarin (AMCA), Eosin, Erythrosin, BODIPY®, Cascade Blue®, Oregon Green®, pyrene, lissamine, xanthenes, acridines, oxazines, phycoerythrin, macrocyclic chelates of lanthanide ions such as quantum dye™, fluorescent energy transfer dyes, such as thiazole orange-ethidium heterodimer, and the
10   cyanine dyes Cy3, Cy3.5, Cy5, Cy5.5 and Cy7. Examples of other specific fluorescent labels include 3-Hydroxypyrene 5,8,10-Tri Sulfonic acid, 5-Hydroxy Tryptamine (5-HT), Acid Fuchsin, Alizarin Complexon, Alizarin Red, Allophycocyanin, Aminocoumarin, Anthroyl Stearate, Astrazon Brilliant Red 4G, Astrazon Orange R, Astrazon Red 6B, Astrazon Yellow 7 GLL, Atabrine, Auramine, Aurophosphine, Aurophosphine G, BAO 9
15   (Bisaminophenyloxadiazole), BCECF, Berberine Sulphate, Bisbenzamide, Blancophor FFG Solution, Blancophor SV, Bodipy F1, Brilliant Sulphoflavin FF, Calcien Blue, Calcium Green, Calcofluor RW Solution, Calcofluor White, Calcophor White ABT Solution, Calcophor White Standard Solution, Carbostyryl, Cascade Yellow, Catecholamine, Chinacrine, Coriphosphine O, Coumarin-Phalloidin, CY3.1 8, CY5.1 8, CY7, Dans (1-
20   Dimethyl Amino Naphaline 5 Sulphonic Acid), Dansa (Diamino Naphtyl Sulphonic Acid), Dansyl NH-CH3, Diamino Phenyl Oxydiazole (DAO), Dimethylamino-5-Sulphonic acid, Dipyrrometheneboron Difluoride, Diphenyl Brilliant Flavine 7GFF, Dopamine, Erythrosin ITC, Euchrysin, FIF (Formaldehyde Induced Fluorescence), Flazo Orange, Fluo 3, Fluorescamine, Fura-2, Genacryl Brilliant Red B, Genacryl Brilliant Yellow 10GF,
25   Genacryl Pink 3G, Genacryl Yellow 5GF, Gloxalic Acid, Granular Blue, Haematoporphyrin, Indo-1, Intrawhite Cf Liquid, Leucophor PAF, Leucophor SF, Leucophor WS, Lissamine Rhodamine B200 (RD200), Lucifer Yellow CH, Lucifer Yellow VS, Magdala Red, Marina Blue, Maxilon Brilliant Flavin 10 GFF, Maxilon Brilliant Flavin 8 GFF, MPS (Methyl Green Pyronine Stilbene), Mithramycin, NBD Amine,
30   Nitrobenzoxadidole, Noradrenaline, Nuclear Fast Red, Nuclear Yellow, Nylosan Brilliant Flavin E8G, Oxadiazole, Pacific Blue, Pararosaniline (Feulgen), Phorwite AR Solution, Phorwite BKL, Phorwite Rev, Phorwite RPA, Phosphine 3R, Phthalocyanine, Phycoerythrin R, Polyazaindacene Pontochrome Blue Black, Porphyrin, Primuline, Procion

Yellow, Pyronine, Pyronine B, Pyrozal Brilliant Flavin 7GF, Quinacrine Mustard,

Rhodamine 123, Rhodamine 5 GLD, Rhodamine 6G, Rhodamine B, Rhodamine B 200,

Rhodamine B Extra, Rhodamine BB, Rhodamine BG, Rhodamine WT, Serotonin, Sevron

Brilliant Red 2B, Sevron Brilliant Red 4G, Sevron Brilliant Red B, Sevron Orange, Sevron

5    Yellow L, SITS (Primuline), SITS (Stilbene Isothiosulphonic acid), Stilbene, Snarf 1,

sulpho Rhodamine B Can C, Sulpho Rhodamine G Extra, Tetracycline, Thiazine Red R,

Thioflavin S, Thioflavin TCN, Thioflavin 5, Thiolyte, Thiozol Orange, Tinopol CBS, True

Blue, Ultralite, Uranine B, Uvitex SFC, Xylene Orange, and XRITC.

Preferred fluorescent labels are fluorescein (5-carboxyfluorescein-N-

10   hydroxysuccinimide ester), rhodamine (5,6-tetramethyl rhodamine), and the cyanine dyes

Cy3, Cy3.5, Cy5, Cy5.5 and Cy7.  The absorption and emission maxima, respectively, for

these fluors are: FITC (490 nm; 520 nm), Cy3 (554 nm; 568 nm), Cy3.5 (581 nm; 588 nm),

Cy5 (652 nm: 672 nm), Cy5.5 (682 nm; 703 nm) and Cy7 (755 nm; 778 nm), thus allowing

their simultaneous detection.  Other examples of fluorescein dyes include 6-

15   carboxyfluorescein (6-FAM), 2',4',1,4,-tetrachlorofluorescein (TET), 2',4',5',7',1,4-

hexachlorofluorescein (HEX), 2',7'-dimethoxy-4', 5'-dichloro-6-carboxyrhodamine (JOE),

2'-chloro-5'-fluoro-7',8'-fused phenyl-1,4-dichloro-6-carboxyfluorescein (NED), and 2'-

chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC).  Fluorescent labels can be

obtained from a variety of commercial sources, including Amersham Pharmacia Biotech,

20   Piscataway, NJ; Molecular Probes, Eugene, OR; and Research Organics, Cleveland, Ohio.

Additional labels of interest include those that provide for signal only when the

probe with which they are associated is specifically bound to a target molecule, where such

labels include: "molecular beacons" as described in Tyagi & Kramer, Nature Biotechnology

(1996) 14:303 and EP 0 070 685 B1.  Other labels of interest include those described in

25   U.S. Pat. No. 5,563,037; WO 97/17471 and WO 97/17076.

Labeled nucleotides are a preferred form of detection label since they can be directly

incorporated into the amplification products during synthesis.  Examples of detection labels

that can be incorporated into amplified nucleic acids include nucleotide analogs such as

BrdUrd (5-bromodeoxyuridine, Hoy and Schimke, *Mutation Research* **290**:217-230

30   (1993)), aminoallyldeoxyuridine (Henegariu *et al., Nature Biotechnology* **18**:345-348

(2000)), 5-methylcytosine (Sano *et al., Biochim. Biophys. Acta* **951**:157-165 (1988)),

bromouridine (Wansick *et al., J. Cell Biology* **122**:283-293 (1993)) and nucleotides

modified with biotin (Langer *et al., Proc. Natl. Acad. Sci. USA* **78**:6633 (1981)) or with

suitable haptens such as digoxygenin (Kerkhof, *Anal. Biochem.* 205:359-364 (1992)). Suitable fluorescence-labeled nucleotides are Fluorescein-isothiocyanate-dUTP, Cyanine-3-dUTP and Cyanine-5-dUTP (Yu *et al., Nucleic Acids Res.*, 22:3226-3232 (1994)). A preferred nucleotide analog detection label for DNA is BrdUrd (bromodeoxyuridine,

5      BrdUrd, BrdU, BUdR, Sigma-Aldrich Co). Other preferred nucleotide analogs for incorporation of detection label into DNA are AA-dUTP (aminoallyl-deoxyuridine triphosphate, Sigma-Aldrich Co.), and 5-methyl-dCTP (Roche Molecular Biochemicals). A preferred nucleotide analog for incorporation of detection label into RNA is biotin-16-UTP (biotin-16-uridine-5'-triphosphate, Roche Molecular Biochemicals). Fluorescein, Cy3, and

10     Cy5 can be linked to dUTP for direct labelling. Cy3.5 and Cy7 are available as avidin or anti-digoxygenin conjugates for secondary detection of biotin- or digoxygenin-labelled probes.

           Detection labels that are incorporated into amplified nucleic acid, such as biotin, can be subsequently detected using sensitive methods well-known in the art. For example,

15     biotin can be detected using streptavidin-alkaline phosphatase conjugate (Tropix, Inc.), which is bound to the biotin and subsequently detected by chemiluminescence of suitable substrates (for example, chemiluminescent substrate CSPD: disodium, 3-(4-methoxyspiro-[1,2,-dioxetane-3-2'-(5'-chloro)tricyclo [3.3.1.1$^{3,7}$]decane]-4-yl) phenyl phosphate; Tropix, Inc.). Labels can also be enzymes, such as alkaline phosphatase, soybean peroxidase,

20     horseradish peroxidase and polymerases, that can be detected, for example, with chemical signal amplification or by using a substrate to the enzyme which produces light (for example, a chemiluminescent 1,2-dioxetane substrate) or fluorescent signal.

           Molecules that combine two or more of these detection labels are also considered detection labels. Any of the known detection labels can be used with the disclosed probes,

25     tags, and method to label and detect nucleic acid amplified using the disclosed method. Methods for detecting and measuring signals generated by detection labels are also known to those of skill in the art. For example, radioactive isotopes can be detected by scintillation counting or direct visualization; fluorescent molecules can be detected with fluorescent spectrophotometers; phosphorescent molecules can be detected with a spectrophotometer or

30     directly visualized with a camera; enzymes can be detected by detection or visualization of the product of a reaction catalyzed by the enzyme; antibodies can be detected by detecting a secondary detection label coupled to the antibody. As used herein, detection molecules are

molecules which interact with amplified nucleic acid and to which one or more detection labels are coupled.

**M. Detection Probes**

5 Detection probes are labeled oligonucleotides having sequence complementary to detection tags on amplified nucleic acids. The complementary portion of a detection probe can be any length that supports specific and stable hybridization between the detection probe and the detection tag. For this purpose, a length of 10 to 35 nucleotides is preferred, with a complementary portion of a detection probe 16 to 20 nucleotides long being most preferred. Detection probes can contain any of the detection labels described above.

10 Preferred labels are biotin and fluorescent molecules. A particularly preferred detection probe is a molecular beacon. Molecular beacons are detection probes labeled with fluorescent moieties where the fluorescent moieties fluoresce only when the detection probe is hybridized (Tyagi and Kramer, *Nature Biotechnol.* **14**:303-309 (1995)). The use of such probes eliminates the need for removal of unhybridized probes prior to label detection

15 because the unhybridized detection probes will not produce a signal. This is especially useful in multiplex assays.

**N. Address Probes**

An address probe is an oligonucleotide having a sequence complementary to address tags on primers. The complementary portion of an address probe can be any length that

20 supports specific and stable hybridization between the address probe and the address tag. For this purpose, a length of 10 to 35 nucleotides is preferred, with a complementary portion of an address probe 12 to 18 nucleotides long being most preferred. An address probe can contain a single complementary portion or multiple complementary portions. Preferably, address probes are coupled, either directly or via a spacer molecule, to a solid-state support.

25 Such a combination of address probe and solid-state support are a preferred form of solid-state detector.

**O. Oligonucleotide Synthesis**

Primers, detection probes, address probes, and any other oligonucleotides can be synthesized using established oligonucleotide synthesis methods. Methods to produce or

30 synthesize oligonucleotides are well known in the art. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) Chapters 5, 6) to purely

synthetic methods, for example, by the cyanoethyl phosphoramidite method. Solid phase chemical synthesis of DNA fragments is routinely performed using protected nucleoside cyanoethyl phosphoramidites (S. L. Beaucage et al. (1981) Tetrahedron Lett. 22:1859). In this approach, the 3'-hydroxyl group of an initial 5'-protected nucleoside is first covalently attached to the polymer support (R. C. Pless et al. (1975) Nucleic Acids Res. 2:773 (1975)). Synthesis of the oligonucleotide then proceeds by deprotection of the 5'-hydroxyl group of the attached nucleoside, followed by coupling of an incoming nucleoside-3'-phosphoramidite to the deprotected hydroxyl group (M. D. Matteucci et a. (1981) J. Am. Chem. Soc. 103:3185). The resulting phosphite triester is finally oxidized to a phosphorotriester to complete the internucleotide bond (R. L. Letsinger et al. (1976) J. Am. Chem. Soc. 9:3655). Alternatively, the synthesis of phosphorothioate linkages can be carried out by sulfurization of the phosphite triester. Several chemicals can be used to perform this reaction, among them 3H-1,2-benzodithiole-3-one, 1,1-dioxide (R.P. Iyer, W. Egan, J.B. Regan, and S.L. Beaucage, J. Am. Chem. Soc., 1990, 112, 1253-1254). The steps of deprotection, coupling and oxidation are repeated until an oligonucleotide of the desired length and sequence is obtained. Other methods exist to generate oligonucleotides such as the H-phosphonate method (Hall et al, (1957) J. Chem. Soc., 3291-3296) or the phosphotriester method as described by Ikuta *et al.*, *Ann. Rev. Biochem.* **53**:323-356 (1984), (phosphotriester and phosphite-triester methods), and Narang *et al.*, *Methods Enzymol.*, **65**:610-620 (1980), (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen *et al.*, *Bioconjug. Chem.* **5**:3-7 (1994). Other forms of oligonucleotide synthesis are described in U.S. Patent No. 6,294,664 and U.S. Patent No. 6,291,669.

The nucleotide sequence of an oligonucleotide is generally determined by the sequential order in which subunits of subunit blocks are added to the oligonucleotide chain during synthesis. Each round of addition can involve a different, specific nucleotide precursor, or a mixture of one or more different nucleotide precursors. For the disclosed primers of specific sequence, specific nucleotide precursors would be added sequentially. In general, degenerate or random positions in an oligonucleotide can be produced by using a mixture of nucleotide precursors representing the range of nucleotides that can be present at that position. Thus, precursors for A and T can be included in the reaction for a particular position in an oligonucleotide if that position is to be degenerate for A and T. Precursors for all four nucleotides can be included for a fully degenerate or random position.

Completely random oligonucleotides can be made by including all four nucleotide precursors in every round of synthesis. Degenerate oligonucleotides can also be made having different proportions of different nucleotides. Such oligonucleotides can be made,

5      for example, by using different nucleotide precursors, in the desired proportions, in the reaction.

Many of the oligonucleotides described herein are designed to be complementary to certain portions of other oligonucleotides or nucleic acids such that stable hybrids can be formed between them. The stability of these hybrids can be calculated using known methods such as those described in Lesnick and Freier, *Biochemistry* 34:10807-10815

10     (1995), McGraw *et al.*, *Biotechniques* 8:674-678 (1990), and Rychlik *et al.*, *Nucleic Acids Res.* 18:6409-6412 (1990).

Hexamer oligonucleotides were synthesized on a Perseptive Biosystems 8909 Expedite Nucleic Acid Synthesis system using standard β-cyanoethyl phosphoramidite coupling chemistry on mixed dA+dC+dG+dT synthesis columns (Glen Research, Sterling,

15     VA). The four phosphoramidites were mixed in equal proportions to randomize the bases at each position in the oligonucleotide. Oxidation of the newly formed phosphites were carried out using the sulfurizing reagent 3H-1,2-benzothiole-3-one-1,1-idoxide (Glen Research) instead of the standard oxidizing reagent after the first and second phosphoramidite addition steps. The thio-phosphitylated oligonucleotides were deprotected using 30% ammonium

20     hydroxide (3.0 ml) in water at 55°C for 16 hours, concentrated in an OP 120 Savant Oligo Prep deprotection unit for 2 hours, and desalted with PD10 Sephadex columns using the protocol provided by the manufacturer.

So long as their relevant function is maintained, primers, detection probes, address probes, and any other oligonucleotides can be made up of or include modified nucleotides

25     (nucleotide analogs). Many modified nucleotides are known and can be used in oligonucleotides. A nucleotide analog is a nucleotide which contains some type of modification to either the base, sugar, or phosphate moieties. Modifications to the base moiety would include natural and synthetic modifications of A, C, G, and T/U as well as different purine or pyrimidine bases, such as uracil-5-yl, hypoxanthin-9-yl (I), and

30     2-aminoadenin-9-yl. A modified base includes but is not limited to 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and

cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine,

5      8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.  Additional base modifications can be found for example in U.S. Pat. No. 3,687,808, Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and Sanghvi, Y. S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B. ed., CRC Press, 1993.  Certain nucleotide analogs, such as

10     5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine can increase the stability of duplex formation.  Other modified bases are those that function as universal bases.  Universal bases include 3-nitropyrrole and 5-nitroindole.  Universal bases substitute for the normal bases but have no bias in base

15     pairing.  That is, universal bases can base pair with any other base.  Base modifications often can be combined with for example a sugar modification, such as 2'-O-methoxyethyl, to achieve unique properties such as increased duplex stability.  There are numerous United States patents such as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121,

20     5,596,091; 5,614,617; and 5,681,941, which detail and describe a range of base modifications.  Each of these patents is herein incorporated by reference in its entirety, and specifically for their description of base modifications, their synthesis, their use, and their incorporation into oligonucleotides and nucleic acids.

Nucleotide analogs can also include modifications of the sugar moiety.

25     Modifications to the sugar moiety would include natural modifications of the ribose and deoxyribose as well as synthetic modifications.  Sugar modifications include but are not limited to the following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C1 to C10, alkyl or C2 to C10 alkenyl and

30     alkynyl.  2' sugar modifications also include but are not limited to $-O[(CH_2)_n O]_m CH_3$, $-O(CH_2)_n OCH_3$, $-O(CH_2)_n NH_2$, $-O(CH_2)_n CH_3$, $-O(CH_2)_n -ONH_2$, and $-O(CH_2)_n ON[(CH_2)_n CH_3)]_2$, where n and m are from 1 to about 10.

Other modifications at the 2' position include but are not limited to: C1 to C10 lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH$_3$, OCN, Cl, Br, CN, CF$_3$, OCF$_3$, SOCH$_3$, SO$_2$ CH$_3$, ONO$_2$, NO$_2$, N$_3$, NH$_2$, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving

5    group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications may also be made at other positions on the sugar, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5'

10   position of 5' terminal nucleotide. Modified sugars would also include those that contain modifications at the bridging ring oxygen, such as CH$_2$ and S. Nucleotide sugar analogs may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. There are numerous United States patents that teach the preparation of such modified sugar structures such as 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137;

15   5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety, and specifically for their description of modified sugar structures, their synthesis, their use, and their incorporation into nucleotides, oligonucleotides and nucleic acids.

20   Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include but are not limited to those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates,

25   phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. It is understood that these phosphate or modified phosphate linkages between two nucleotides can be through a 3'-5' linkage or a 2'-5' linkage, and the linkage can contain inverted polarity such as 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts

30   and free acid forms are also included. Numerous United States patents teach how to make and use nucleotides containing modified phosphates and include but are not limited to, 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677;

5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference its entirety, and specifically for their description of modified phosphates, their synthesis, their use, and their incorporation into nucleotides, oligonucleotides and nucleic acids.

It is understood that nucleotide analogs need only contain a single modification, but may also contain multiple modifications within one of the moieties or between different moieties.

Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize and hybridize to (base pair to) complementary nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

Nucleotide substitutes are nucleotides or nucleotide analogs that have had the phosphate moiety and/or sugar moieties replaced. Nucleotide substitutes do not contain a standard phosphorus atom. Substitutes for the phosphate can be for example, short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH2 component parts. Numerous United States patents disclose how to make and use these types of phosphate replacements and include but are not limited to 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference its entirety, and specifically for their description of phosphate replacements, their synthesis, their use, and their incorporation into nucleotides, oligonucleotides and nucleic acids.

It is also understood in a nucleotide substitute that both the sugar and the phosphate moieties of the nucleotide can be replaced, by for example an amide type linkage (aminoethylglycine) (PNA). United States patents 5,539,082; 5,714,331; and 5,719,262 teach how to make and use PNA molecules, each of which is herein incorporated by

5      reference. (See also Nielsen *et al.*, *Science* 254:1497-1500 (1991)).

Oligonucleotides can be comprised of nucleotides and can be made up of different types of nucleotides or the same type of nucleotides. For example, one or more of the nucleotides in an oligonucleotide can be ribonucleotides, 2'-O-methyl ribonucleotides, or a mixture of ribonucleotides and 2'-O-methyl ribonucleotides; about 10% to about 50% of the

10     nucleotides can be ribonucleotides, 2'-O-methyl ribonucleotides, or a mixture of ribonucleotides and 2'-O-methyl ribonucleotides; about 50% or more of the nucleotides can be ribonucleotides, 2'-O-methyl ribonucleotides, or a mixture of ribonucleotides and 2'-O-methyl ribonucleotides; or all of the nucleotides are ribonucleotides, 2'-O-methyl ribonucleotides, or a mixture of ribonucleotides and 2'-O-methyl ribonucleotides. Such

15     oligonucleotides can be referred to as chimeric oligonucleotides.

**P.  DNA polymerases**

DNA polymerases useful in multiple displacement amplification must be capable of displacing, either alone or in combination with a compatible strand displacement factor, a hybridized strand encountered during replication. Such polymerases are referred to herein

20     as strand displacement DNA polymerases. It is preferred that a strand displacement DNA polymerase lack a 5' to 3' exonuclease activity. Strand displacement is necessary to result in synthesis of multiple copies of a target sequence. A 5' to 3' exonuclease activity, if present, might result in the destruction of a synthesized strand. It is also preferred that DNA polymerases for use in the disclosed method are highly processive. The suitability of a

25     DNA polymerase for use in the disclosed method can be readily determined by assessing its ability to carry out strand displacement replication. Preferred strand displacement DNA polymerases are bacteriophage φ29 DNA polymerase (U.S. Patent Nos. 5,198,543 and 5,001,050 to Blanco *et al.*), Bst large fragment DNA polymerase (Exo(-) Bst; Aliotta *et al.*, *Genet. Anal. (Netherlands)* 12:185-195 (1996)) and exo(-)Bca DNA polymerase (Walker

30     and Linn, *Clinical Chemistry* 42:1604-1608 (1996)). Other useful polymerases include phage M2 DNA polymerase (Matsumoto *et al.*, *Gene* 84:247 (1989)), phage φPRD1 DNA polymerase (Jung *et al.*, *Proc. Natl. Acad. Sci. USA* 84:8287 (1987)), exo(-)VENT® DNA polymerase (Kong *et al.*, *J. Biol. Chem.* 268:1965-1975 (1993)), Klenow fragment of DNA

90

polymerase I (Jacobsen *et al.*, *Eur. J. Biochem.* **45**:623-627 (1974)), T5 DNA polymerase
(Chatterjee *et al.*, *Gene* **97**:13-19 (1991)), Sequenase (U.S. Biochemicals), PRD1 DNA
polymerase (Zhu and Ito, *Biochim. Biophys. Acta.* **1219**:267-276 (1994)), and T4 DNA
polymerase holoenzyme (Kaboord and Benkovic, *Curr. Biol.* **5**:149-157 (1995)).  φ29 DNA

5      polymerase is most preferred.

       As used herein, a thermolabile nucleic acid polymerase is a nucleic acid polymerase
that is notably inactivated at the temperature at which an amplification reaction is carried
out in the absence of an additive, dNTPs, and template nucleic acid.  Thus, whether a
nucleic acid polymerase is thermolabile depends on the temperature at which an

10     amplification reaction is carried out.  Note that as used herein, thermolability does not
require denaturation or irreversible inactivation of a polymerase.  All that is required is that
the polymerase be notably incapable of performing template-dependent polymerization at
the temperature at which an amplification reaction is carried out in the absence of an
additive.

15     As used herein, an elevated temperature is a temperature at or above which a given
nucleic acid polymerase is notably inactivated in the absence of an additive, dNTPs, and
template nucleic acid.  Thus, what constitutes an elevated temperature depends on the
particular nucleic acid polymerase.  As used herein, notable inactivation refers to a
reduction in activity of 40% or more.  Substantial inactivation refers to a reduction in

20     activity of 60% or more.  Significant inactivation refers to a reduction in activity of 80% or
more.

       Strand displacement can be facilitated through the use of a strand displacement
factor, such as helicase.  It is considered that any DNA polymerase that can perform strand
displacement replication in the presence of a strand displacement factor is suitable for use in

25     the disclosed method, even if the DNA polymerase does not perform strand displacement
replication in the absence of such a factor.  Strand displacement factors useful in strand
displacement replication include BMRF1 polymerase accessory subunit (Tsurumi *et al.*, *J.
Virology* **67**(12):7648-7653 (1993)), adenovirus DNA-binding protein (Zijderveld and van
der Vliet, *J. Virology* **68**(2):1158-1164 (1994)), herpes simplex viral protein ICP8 (Boehmer

30     and Lehman, *J. Virology* **67**(2):711-715 (1993); Skaliter and Lehman, *Proc. Natl. Acad. Sci.
USA* **91**(22):10665-10669 (1994)); single-stranded DNA binding proteins (SSB; Rigler and
Romano, *J. Biol. Chem.* **270**:8910-8919 (1995)); phage T4 gene 32 protein (Villemain and

Giedroc, *Biochemistry* **35**:14395-14404 (1996); and calf thymus helicase (Siegel *et al.*, *J. Biol. Chem.* **267**:13629-13635 (1992)).

The ability of a polymerase to carry out strand displacement replication can be determined by using the polymerase in a strand displacement replication assay such as those
5      described in Examples 4 and 8. The assay in the examples can be modified as appropriate. For example, a helicase can be used instead of SSB. Such assays should be performed at a temperature suitable for optimal activity for the enzyme being used, for example, 32°C for φ29 DNA polymerase, from 46°C to 64°C for exo(-) Bst DNA polymerase, or from about 60°C to 70°C for an enzyme from a hyperthermophylic organism. For assays from 60°C to
10     70°C, primer length may be increased to provide a melting temperature appropriate for the assay temperature. Another useful assay for selecting a polymerase is the primer-block assay described in Kong *et al.*, *J. Biol. Chem.* **268**:1965-1975 (1993). The assay consists of a primer extension assay using an M13 ssDNA template in the presence or absence of an oligonucleotide that is hybridized upstream of the extending primer to block its progress.
15     Enzymes able to displace the blocking primer in this assay are expected to be useful for the disclosed method.

## Q. Kits

The materials described above can be packaged together in any suitable combination as a kit useful for performing the disclosed method. Kit components in a given kit can be
20     designed and adapted for use together in the disclosed method. For example, disclosed are kits for amplifying genomic DNA, the kit comprising a lysis solution, a stabilization solution, a set of primers, and a DNA polymerase. The components of such a kit are described elsewhere herein. In some forms of the kit, the lysis solution can comprise potassium hydroxide, for example, 400 mM KOH. Some useful forms of lysis solution can
25     comprise 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA. In some forms of the kit, the stabilization solution can comprise Tris-HCl at pH 4.1. Some useful forms of stabilization solution can comprise 800 mM Tris-HCl, pH 4.1. In some forms of the kit, the set of primers can comprise random hexamer primers. In some forms of the kit, the DNA polymerase can be φ29 DNA polymerase. In some forms of the kit, the kit can further
30     comprise deoxynucleotide triphosphates. In some forms of the kit, the kit can further comprise one or more detection probes. Detection probes are described elsewhere herein. In some forms of the kit, the detection probes can each comprise a complementary portion, where the complementary portion is complementary to a nucleic acid sequence of interest.

92

In some forms of the kit, the kit can further comprise denaturing solution. In some forms of the kit, the kit can further comprise reaction mix. Also disclosed are kits for amplification of nucleic acid samples, the kit comprising a single primer and φ29 DNA polymerase. The kits also can contain nucleotides, buffers, detection probes, fluorescent change probes, lysis

5    solutions, stabilization solutions, denaturation solutions, or a combination.

Some useful kits can comprise a lysis solution, a stabilization solution, a set of primers, a φ29 DNA polymerase, 1M dithiotheitol, 1X Phosphase-Buffered Saline, pH 7.5, and control DNA template; where the lysis solution comprises 400 mM KOH and 10 mM EDTA, the stabilization solution comprises 800 mM Tris-HCl, pH 4, and the set of primers

10   comprises a reaction mix; where the reaction mix comprises 150 mM Tris-HCl, 200 mM KCl, 40 mM MgCl$_2$, 20 mM (NH$_4$)$_2$SO$_4$, 4 mM deoxynucleotide triphosphates, and 0.2 mM random hexamer primers.

The nucleic acid polymerase can be Phi29 DNA polymerase. The additive can be a sugar, a chaperone, a protein, trehalose, glucose, sucrose, or a combination. The additive

15   can comprise trehalose, the set of primers can comprise exonuclease-resistant random hexamer primers, and the nucleic acid polymerase can comprise Phi29 DNA polymerase. The kit can further comprise one or more components that, when mixed in appropriate amounts, produce a reaction mixture having final concentrations of 10 mM MgCl$_2$, 37.5 mM Tris-HCl, pH 7, 50 mM KCl, 20 mM Ammonium Sulfate, and 1 mM dNTPs. The kit

20   can further comprise any one or a combination of a stabilization solution, a lysis solution, a reaction mix that comprises the set of primers, dithiotheitol, Phosphate-Buffered Saline, and control DNA template. The stabilization solution can comprise 800 mM Tris-HCl, pH 4; the lysis solution can comprise 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA; the reaction mix can comprise 150 mM Tris-HCl, 200 mM KCl, 40 mM MgCl$_2$, 20 mM

25   (NH$_4$)$_2$SO$_4$, 4 mM deoxynucleotide triphosphates, and 0.2 mM random hexamer primers; the dithiothreitol can comprise 1M dithiotheitol; and the Phosphate-Buffered Saline can comprise 1X Phosphate-Buffered Saline, pH 7.5. The components of such kits are described elsewhere herein.

Any of the components that can be present in a kit that can be used together can be

30   combined in a single component of the kit. Thus, a reaction mix can include, for example, buffers, deoxynucleotide triphosphates and primers. Similarly, components and solutions can be divided into constituent parts or sub-solutions. The kits can be used for any purpose, generally for nucleic acid amplification. In some forms, the kit can be designed to detect

nucleic acid sequences of interest in a genome or other nucleic acid sample. In some forms, the kit can be designed to assess a disease, condition or predisposition of an individual based on a nucleic acid sequences of interest.

**R. Mixtures**

5      Disclosed are mixtures formed by performing, or formed during the course of performing, any form of the disclosed method. For example, disclosed are mixtures comprising, for example, cells and lysis solution; cell lysate and stabilization solution; stabilized cell lysate and one or more primers; stabilized cell lysate and DNA polymerase; stabilized cell lysate, one or more primers, and DNA polymerase; stabilized cell lysate and

10     replicated strands; stabilized cell lysate, one or more primers, and replicated strands; stabilized cell lysate, DNA polymerase, and replicated strands; stabilized cell lysate, one or more primers, DNA polymerase, and replicated strands; stabilized cell lysate and one or more detection probes; stabilized cell lysate, one or more primers, one or more detection probes, and replicated strands; stabilized cell lysate, DNA polymerase, one or more

15     detection probes, and replicated strands; stabilized cell lysate, one or more primers, DNA polymerase, one or more detection probes, and replicated strands, sample and lysis solution; sample and stabilization solution; stabilized sample and one or more primers; stabilized sample and DNA polymerase; stabilized sample, one or more primers, and DNA polymerase; stabilized sample and replicated strands; stabilized sample, one or more

20     primers, and replicated strands; stabilized sample, DNA polymerase, and replicated strands; stabilized sample, one or more primers, DNA polymerase, and replicated strands; stabilized sample and one or more detection probes; stabilized sample, one or more primers, one or more detection probes, and replicated strands; stabilized sample, DNA polymerase, one or more detection probes, and replicated strands; and stabilized sample, one or more primers,

25     DNA polymerase, one or more detection probes, and replicated strands.

       Also disclosed are mixtures comprising a single primer, a nucleic acid sample, and a DNA polymerase; a single primer, a genomic nucleic acid sample, and a DNA polymerase; one or more primers, one or more nucleic acid samples, and one or more DNA polymerases; a single primer, a nucleic acid sample, and one or more detection probes; a single primer, a

30     nucleic acid sample, and one or more fluorescent change probes; a single primer, a nucleic acid sample, and replicated nucleic acid molecules; a single primer, a genomic nucleic acid sample, and replicated nucleic acid molecules; one or more primers, one or more nucleic acid samples, and replicated nucleic acid molecules; a single primer, a nucleic acid sample,

replicated nucleic acid molecules, and one or more detection probes; a single primer, a nucleic acid sample, replicated nucleic acid molecules, and one or more fluorescent change probes.

Whenever the method involves mixing or bringing into contact, for example, compositions or components or reagents, performing the method creates a number of different mixtures. For example, if the method includes three mixing steps, after each one of these steps a unique mixture is formed if the steps are performed sequentially. In addition, a mixture is formed at the completion of all of the steps regardless of how the steps were performed. The present disclosure contemplates these mixtures, obtained by the performance of the disclosed method as well as mixtures containing any disclosed reagent, composition, or component, for example, disclosed herein.

## S. Systems

Disclosed are systems useful for performing, or aiding in the performance of, the disclosed method. Systems generally comprise combinations of articles of manufacture such as structures, machines, devices, and the like, and compositions, compounds, materials, and the like. Such combinations that are disclosed or that are apparent from the disclosure are contemplated. For example, disclosed and contemplated are systems comprising solid supports and primers, nucleic acid samples, detection probes, fluorescent change probes, or a combination.

## T. Data Structures and Computer Control

Disclosed are data structures used in, generated by, or generated from, the disclosed method. Data structures generally are any form of data, information, and/or objects collected, organized, stored, and/or embodied in a composition or medium. A nucleic acid library stored in electronic form, such as in RAM or on a storage disk, is a type of data structure.

The disclosed method, or any part thereof or preparation therefor, can be controlled, managed, or otherwise assisted by computer control. Such computer control can be accomplished by a computer controlled process or method, can use and/or generate data structures, and can use a computer program. Such computer control, computer controlled processes, data structures, and computer programs are contemplated and should be understood to be disclosed herein.

## Uses

The disclosed methods and compositions are applicable to numerous areas including, but not limited to, analysis of nucleic acids present in cells (for example, analysis of genomic DNA in cells), disease detection, mutation detection, gene discovery, gene mapping (molecular haplotyping), and agricultural research. Particularly useful is whole genome amplification. Other uses include, for example, detection of nucleic acids in cells and on genomic DNA arrays; molecular haplotyping; mutation detection; detection of inherited diseases such as cystic fibrosis, muscular dystrophy, diabetes, hemophilia, sickle cell anemia; assessment of predisposition for cancers such as prostate cancer, breast cancer, lung cancer, colon cancer, ovarian cancer, testicular cancer, pancreatic cancer.

## Method

Disclosed are methods of amplifying nucleic acids. In particular, disclosed are methods of producing amplification products with low amplification bias and/or other measures of the quality of the amplification products. It has been discovered that amplification reactions can produce amplification products of high quality, such as low amplification bias, if performed on an amount of nucleic acid at or over a threshold amount and/or on nucleic acids at or below a threshold concentration. The threshold amount and concentration can vary depending on the nature and source of the nucleic acids to be amplified and the type of amplification reaction employed. Disclosed is a method of determining the threshold amount and/or threshold concentration of nucleic acids that can be used with nucleic acid samples of interest in amplification reactions of interest. Because amplification reactions can produce high quality amplification products, such as low bias amplification products, below the threshold amount and/or concentration of nucleic acid, such below-threshold amounts and/or concentrations can be used. Accordingly, also disclosed is a method of determining amounts and/or concentrations of nucleic acids that can be used with nucleic acid samples of interest in amplification reactions of interest to produce amplification products having less than a selected amplification bias (or other measure of the quality of the amplified nucleic acids). The quality of the amplification products produced by the disclosed methods can be measured by any desired standard, and the threshold amount (or above) and/or threshold concentration (or below) to achieve a desired level of quality measured by a standard of interest can be determined by, and for used in, the disclosed methods.

It has also been discovered that exposure of nucleic acids to alkaline conditions, reduction of the pH of nucleic acids exposed to alkaline conditions, and incubation of the resulting nucleic acids at or over a threshold amount and/or at or below a threshold concentration can produce amplification products with low amplification bias. Such an

5     alkaline/neutralization procedure can improve the quality of the amplification products. The quality of the amplification products can be measured in a variety of ways, including, but not limited to, amplification bias, allele bias, locus representation, sequence representation, allele representation, locus representation bias, sequence representation bias, percent representation, percent locus representation, percent sequence representation, and

10    other measure that indicate unbiased and/or complete amplification of the input nucleic acids.

Disclosed are methods for amplification of nucleic acid sequences of interest with greater efficiency and fidelity. The disclosed method relates to isothermal amplification techniques, such as Multiple Displacement Amplification (MDA), where the generation of

15    DNA artifacts is decreased or eliminated. Generally, this can be accomplished by carrying out the reaction at elevated temperature. In particularly useful embodiments of the method, sugars and/or other additives can be used to stabilized the polymerase at high temperature.

It has been discovered that generation of high molecular weight artifacts, in an isothermal amplification procedure, is substantially reduced or eliminated while still

20    allowing the desired amplification of input DNA by carrying out the reaction at a higher temperature and, optionally, in the presence of one or more additives. For example, the amplification reaction can be carried out in the presence of sugars at a temperature that is higher then the normal optimal temperature for the DNA polymerase being used. It also has been discovered that isothermal amplification reactions can produce amplification products

25    of high quality, such as low amplification bias, if performed at a higher temperature and, optionally, in the presence of one or more additives.

Disclosed is a method of amplifying nucleic acids, the method comprising incubating nucleic acids comprising target sequences at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an

30    additive, and a set of primers, under conditions promoting replication of the nucleic acids. Replication of the nucleic acids results in replicated strands. During replication at least one of the replicated nucleic acid strands is displaced by strand displacement replication of another replicated strand. Formation of replicated strands from the target sequences is

favored over formation of replicated strands from non-target sequences. Such favored formation can involve any suitable measure of replicated strand formation, such as relative rates of formation, relative amounts of replicated strands formed, amounts of replicated strands formed in comparison to the proportion of the template sequences in the reaction.

5    As one measure, formation of replicated strands from target sequences is favored over formation of replicated strands from non-target sequences when more replicated strands from the target sequences are formed than replicated strands from non-target sequences relative to the proportions of target sequences to non-target sequences present in the reaction. As another measure, formation of replicated strands from target sequences is

10    favored over formation of replicated strands from non-target sequences when the ratio of replicated strands formed from the target sequences to replicated strands formed from non-target sequences increases relative to a standard or control ratio (such as the ratio of the replicated strands observed at non-elevated temperatures).

Also disclosed is a method of amplifying a whole genome, the method comprising

15    exposing cells to alkaline conditions to form a cell lysate, reducing the pH of the cell lysate to form a stabilized cell lysate, and incubating stabilized cell lysate at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, and a set of primers, under conditions promoting replication of the nucleic acids. Replication of the nucleic acids results in replicated

20    strands. During replication at least one of the replicated nucleic acid strands is displaced by strand displacement replication of another replicated strand. Formation of replicated strands from the target sequence is favored over formation of replicated strands from non-target sequences. The cell lysate comprises a whole genome.

Also disclosed is a method of performing strand displacement nucleic acid synthesis

25    at an elevated temperature, the method comprising mixing thermolabile nucleic acid polymerase having strand-displacement activity, nucleic acids comprising target sequences, a set of primers, and an additive, and incubating at an elevated temperature and under conditions favoring hybridization of the primers to the target sequences and extension of the primers by the addition of nucleotides sequentially to the 3' end of the primer in a template-

30    dependent manner, wherein the extension results in replication of the target sequences.

Also disclosed is a method of amplifying a whole genome, the method comprising exposing cells to alkaline conditions to form a cell lysate, wherein the cell lysate comprises a whole genome, reducing the pH of the cell lysate to form a stabilized cell lysate, and

98

incubating stabilized cell lysate at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, a set of primers, and deoxyribonucleotide triphosphates under conditions promoting replication of nucleic acids. During replication at least one of the replicated nucleic acid strands is displaced by

5      strand displacement replication of another replicated strand. Formation of template-dependent extension products in the replication reaction is favored over formation of non-templated product.

Also disclosed is a method of performing strand displacement nucleic acid synthesis at an elevated temperature, the method comprising mixing thermolabile nucleic acid

10     polymerase having strand-displacement activity, single-stranded template nucleic acid, a set of primers, deoxyribonucleotide triphosphates and an additive, and incubating at an elevated temperature and under conditions favoring hybridization of primer to template nucleic acid and extension of primer by the addition of nucleotides sequentially to the 3' end of the primer in a template-dependent manner, wherein said polymerization results in replication

15     of said template nucleic acid.

Also disclosed is a method of amplifying nucleic acids, the method comprising incubating nucleic acids at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, a set of primers, and deoxyribonucleotide triphosphates under conditions promoting replication of nucleic acids.

20     During replication at least one of the replicated nucleic acid strands is displaced by strand displacement replication of another replicated strand. Formation of template-dependent extension products in the replication reaction is favored over formation of non-templated product.

In some forms of the disclosed method, a genomic sample is prepared by exposing

25     the sample to alkaline conditions to denature the nucleic acids in the sample; reducing the pH of the sample to make the pH of the sample compatible with DNA replication; and incubating the sample under conditions that promote replication of the genome. In some embodiments, the conditions of incubation can be conditions that promote replication of the genome and produce amplified genomic nucleic acids having a low amplification bias, an

30     amplification bias at or below a desired level, or any other measure of the quality of the amplification products. Accordingly, also disclosed is a method of determining conditions that can be used with nucleic acid samples of interest in amplification reactions of interest to

produce amplification products having less than a selected amplification bias (or other measure of the quality of the amplified nucleic acids).

In some forms of the disclosed method, a sample that may comprise nucleic acids is exposed to alkaline conditions, where the alkaline conditions promote lysis of cells that may

5    be present in the sample (although the sample need not contain cells), reducing the pH of all or a portion of the sample to form a stabilized sample, and incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample, where the amplification mixture comprises all or a portion of the stabilized sample. Replication of the nucleic acids results in replicated strands, where during replication at

10   least one of the replicated strands is displaced from nucleic acids in the sample by strand displacement replication of another replicated strand, where the replicated strands have low amplification bias. The concentration of nucleic acids in the amplification mixture can be chosen to favor hybridization of primers over reassociation of the nucleic acids. Further, the amount of nucleic acids in the amplification mixture can be at or above a threshold that

15   can result in low amplification bias in the replicated strands.

The disclosed methods can be performed on any desired samples. For example, the disclosed methods can be performed using samples that contain or are suspected of containing nucleic acids. Some forms of the disclosed methods do not require knowledge of any sequence present in a sample in order to amplify nucleic acids in the sample.

20   Accordingly, some forms of the disclosed methods can be used to determine if a sample contains nucleic acids. If amplification products are produced when the method is performed, the sample contains nucleic acids. The disclosed methods can be performed on cells and on nucleic acid samples, including crude nucleic acid samples, partially purified nucleic acid sample, and purified nucleic acid samples. Exposing any cell or nucleic acid

25   sample to alkaline conditions and then reducing the pH of the sample can produce a stabilized sample suitable for amplification or replication.

The disclosed method is based on strand displacement replication of the nucleic acid sequences by multiple primers. The method can be used to amplify one or more specific sequences (multiple strand displacement amplification), an entire genome or other DNA of

30   high complexity (whole genome strand displacement amplification), or concatenated DNA (multiple strand displacement amplification of concatenated DNA). The disclosed method generally involves hybridization of primers to a target nucleic acid sequence and replication of the target sequence primed by the hybridized primers such that replication of the target

sequence results in replicated strands complementary to the target sequence. During replication, the growing replicated strands displace other replicated strands from the target sequence (or from another replicated strand) via strand displacement replication. As used herein, a replicated strand is a nucleic acid strand resulting from elongation of a primer

5      hybridized to a target sequence or to another replicated strand. Strand displacement replication refers to DNA replication where a growing end of a replicated strand encounters and displaces another strand from the template strand (or from another replicated strand). Displacement of replicated strands by other replicated strands is a hallmark of the disclosed method which allows multiple copies of a target sequence to be made in a single, isothermic

10     reaction.

       The disclosed method can accurately and evenly amplify the various sequences in highly complex nucleic acid samples. This result can be quantified by references to, for example, percent representation, sequence representation, sequence representation bias, percent sequence representation, locus representation, locus representation bias, percent

15     locus representation, and/or amplification bias. For example, the replicated nucleic acid molecules produced in the disclosed method can have a sequence representation or sequence representation bias of at least 50% for at least 10 different target sequences. The amplification bias can be less than 10% for at least 10 different target sequences.

       Nucleic acids for amplification are often obtained from cellular samples. This

20     generally requires disruption of the cell (to make the nucleic acid accessible) and purification of the nucleic acids prior to amplification. It also generally requires the inactivation of protein factors such as nucleases that could degrade the DNA, or of factors such as histones that could bind to DNA strands and impede their use as a template for DNA synthesis by a polymerase. There are a variety of techniques used to break open cells,

25     such as sonication, enzymatic digestion of cell walls, heating, and exposure to lytic conditions. Lytic conditions typically involve use of non-physiological pH and/or solvents. Many lytic techniques can result in damage to nucleic acids in cells, including, for example, breakage of genomic DNA. In particular, use of heating to lyse cells can damage genomic DNA and reduce the amount and quality of amplification products of genomic DNA. It has

30     been discovered that alkaline lysis can cause less damage to genomic DNA and can thus result in higher quality amplification results. Alkaline lysis also inactivates protein factors such as nucleases, histones, or other factors that could impede the amplification of DNA within the sample. In addition, it is a useful property of alkaline lysis that reducing the pH

does not reactivate the protein factors, but that such protein factors remain inactivated when the pH of the solution is brought back within a neutral range.

In some forms of the disclosed method, a genomic sample is prepared by exposing cells to alkaline conditions, thereby lysing the cells and resulting in a cell lysate; reducing the pH of the cell lysate to make the pH of the cell lysate compatible with DNA replication; and incubating the cell lysate under conditions that promote replication of the genome of the cells by multiple displacement amplification. Alkaline conditions are conditions where the pH is greater than 9.0. Particularly useful alkaline conditions for the disclosed method are conditions where the pH is greater than 10.0. The alkaline conditions can be, for example, those that cause a substantial number of cells to lyse, those that cause a significant number of cells to lyse, or those that cause a sufficient number of cells to lyse. The number of lysed cells can be considered sufficient if the genome can be sufficiently amplified in the disclosed method. The amplification is sufficient if enough amplification product is produced to permit some use of the amplification product, such as detection of sequences or other analysis. The reduction in pH is generally into the neutral range of pH 9.0 to pH 6.0.

Samples can be exposed to alkaline conditions by mixing the sample with a lysis solution. The amount of lysis solution mixed with the sample can be that amount that causes a substantial denaturation of the nucleic acids in the sample. Generally, this volume will be a function of the pH of the sample/lysis solution mixture. Thus, the amount of lysis solution to mix with a sample can be determined generally from the volume of the sample and the alkaline concentration of the lysis buffer. For example, a smaller volume of a lysis solution with a stronger base and/or higher concentration of base would be needed to create sufficient alkaline conditions than the volume needed of a lysis solution with a weaker base and/or lower concentration of base. The lysis solution can be formulated such that the sample is mixed with an equal volume of the lysis solution (to produce the desired alkaline conditions).

The cells can be exposed to alkaline conditions by mixing the cells with a lysis solution. The amount of lysis solution mixed with the cells can be that amount that causes a substantial number of cells to lyse or those that cause a sufficient number of cells to lyse. Generally, this volume will be a function of the pH of the cell/lysis solution mixture. Thus, the amount of lysis solution to mix with cells can be determined generally from the volume of the cells and the alkaline concentration of the lysis buffer. For example, a smaller volume of a lysis solution with a stronger base and/or higher concentration of base would be

needed to create sufficient alkaline conditions than the volume needed of a lysis solution with a weaker base and/or lower concentration of base. The lysis solution can be formulated such that the cells are mixed with an equal volume of the lysis solution (to produce the desired alkaline conditions).

5          In some embodiments, the lysis solution can comprises a base, such as an aqueous base. Useful bases include potassium hydroxide, sodium hydroxide, potassium acetate, sodium acetate, ammonium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, calcium carbonate, ammonia, aniline, benzylamine, n-butylamine, diethylamine, dimethylamine, diphenylamine, ethylamine,

10         ethylenediamine, methylamine, N-methylaniline, morpholine, pyridine, triethylamine, trimethylamine, aluminum hydroxide, rubidium hydroxide, cesium hydroxide, strontium hydroxide, barium hydroxide, and DBU (1,8-diazobicyclo[5,4,0]undec-7-ene). Useful formulations of lysis solution include lysis solution comprising 400 mM KOH, lysis solution comprising 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA, and lysis

15         solution consisting of 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA.

In some embodiments, the lysis solution can comprise a plurality of basic agents. As used herein, a basic agent is a compound, composition or solution that results in alkaline conditions. In some embodiments, the lysis solution can comprise a buffer. Useful buffers include phosphate buffers, "Good" buffers (such as BES, BICINE, CAPS, EPPS, HEPES,

20         MES, MOPS, PIPES, TAPS, TES, and TRICINE), sodium cacodylate, sodium citrate, triethylammonium acetate, triethylammonium bicarbonate, Tris, Bis-tris, and Bis-tris propane. The lysis solution can comprise a plurality of buffering agents. As used herein, a buffering agent is a compound, composition or solution that acts as a buffer. An alkaline buffering agent is a buffering agent that results in alkaline conditions. In some

25         embodiments, the lysis solution can comprise a combination of one or more bases, basic agents, buffers and buffering agents.

The pH of the cell lysate or sample can be reduced to form a stabilized cell lysate. A stabilized cell lysate or sample is a cell lysate or sample the pH of which is in the neutral range (from about pH 6.0 to about pH 9.0). Useful stabilized cell lysates or samples have a

30         pH that allows replication of nucleic acids in the cell lysate. For example, the pH of the stabilized cell lysate or sample is usefully at a pH at which the DNA polymerase can function. The pH of the cell lysate or sample can be reduced by mixing the cell lysate or sample with a stabilization solution. The stabilization solution comprises a solution that can

reduce the pH of a cell lysate or sample exposed to alkaline conditions as described elsewhere herein.

The amount of stabilization solution mixed with the sample can be that amount that causes a reduction in pH to the neutral range (or other desired pH value). Generally, this

5    volume will be a function of the pH of the sample/stabilization solution mixture. Thus, the amount of stabilization solution to mix with the sample can be determined generally from the volume of the sample, its pH and buffering capacity, and the acidic concentration of the stabilization buffer. For example, a smaller volume of a stabilization solution with a stronger acid and/or higher concentration of acid would be needed to reduce the pH

10   sufficiently than the volume needed of a stabilization solution with a weaker acid and/or lower concentration of acid. The stabilization solution can be formulated such that the sample is mixed with an equal volume of the stabilization solution (to produce the desired pH).

The amount of stabilization solution mixed with the cell lysate can be that amount

15   that causes a reduction in pH to the neutral range (or other desired pH value). Generally, this volume will be a function of the pH of the cell lysate/stabilization solution mixture. Thus, the amount of stabilization solution to mix with the cell lysate can be determined generally from the volume of the cell lysate, its pH and buffering capacity, and the acidic concentration of the stabilization buffer. For example, a smaller volume of a stabilization

20   solution with a stronger acid and/or higher concentration of acid would be needed to reduce the pH sufficiently than the volume needed of a stabilization solution with a weaker acid and/or lower concentration of acid. The stabilization solution can be formulated such that the cell lysate is mixed with an equal volume of the stabilization solution (to produce the desired pH).

25   In some embodiments, the stabilization solution can comprise an acid. Useful acids include hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid, acetylsalicylic acid, ascorbic acid, carbonic acid, citric acid, formic acid, nitric acid, perchloric acid, HF, HBr, HI, $H_2S$, HCN, HSCN, HClO, monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, and any carboxylic acid (ethanoic, propanoic, butanoic, etc., including both linear or

30   branched chain carboxylic acids). In some embodiments, the stabilization solution can comprise a buffer. Useful buffers include Tris-HCl, HEPES, "Good" buffers (such as BES, BICINE, CAPS, EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES, and TRICINE), sodium cacodylate, sodium citrate, triethylammonium acetate, triethylammonium bicarbonate, Tris,

Bis-tris, and Bis-tris propane. Useful formulations of stabilization solutions include stabilization solution comprising 800 mM Tris-HCl; stabilization solution comprising 800 mM Tris-HCl at pH 4.1; and stabilization solution consisting of 800 mM Tris-HCl, pH 4.1.

5          In some embodiments, the stabilization solution can comprise a plurality of acidic agents. As used herein, an acidic agent is a compound, composition or solution that forms an acid in solution. In some embodiments, the stabilization solution can comprise a plurality of buffering agents. An acidic buffering agent is a buffering agent that forms an acid in solution. In some embodiments, the stabilization solution can comprise a combination of one or more acids, acidic agents, buffers and buffering agents.

10         In some embodiments, the pH of the cell lysate or sample can be reduced to about pH 9.0 or below, to about pH 8.5 or below, to about pH 8.0 or below, to about pH 7.5 or below, to about pH 7.2 or below, or to about pH 7.0 or below. In some embodiments, the pH of the cell lysate or sample can be reduced to the range of about pH 9.0 to about pH 6.0, to the range of about pH 9.0 to about pH 6.5, to the range of about pH 9.0 to about pH 6.8,

15         to the range of about pH 9.0 to about pH 7.0, to the range of about pH 9.0 to about pH 7.2, to the range of about pH 9.0 to about pH 7.5, to the range of about pH 9.0 to about pH 8.0, to the range of about pH 9.0 to about pH 8.5, to the range of about pH 8.5 to about pH 6.0, to the range of about pH 8.5 to about pH 6.5, to the range of about pH 8.5 to about pH 6.8, to the range of about pH 8.5 to about pH 7.0, to the range of about pH 8.5 to about pH 7.2,

20         to the range of about pH 8.5 to about pH 7.5, to the range of about pH 8.5 to about pH 8.0, to the range of about pH 8.0 to about pH 6.0, to the range of about pH 8.0 to about pH 6.5, to the range of about pH 8.0 to about pH 6.8, to the range of about pH 8.0 to about pH 7.0, to the range of about pH 8.0 to about pH 7.2, to the range of about pH 8.0 to about pH 7.5, to the range of about pH 7.5 to about pH 6.0, to the range of about pH 7.5 to about pH 6.5,

25         to the range of about pH 7.5 to about pH 6.8, to the range of about pH 7.5 to about pH 7.0, to the range of about pH 7.5 to about pH 7.2, to the range of about pH 7.2 to about pH 6.0, to the range of about pH 7.2 to about pH 6.5, to the range of about pH 7.2 to about pH 6.8, to the range of about pH 7.2 to about pH 7.0, to the range of about pH 7.0 to about pH 6.0, to the range of about pH 7.0 to about pH 6.5, to the range of about pH 7.0 to about pH 6.8,

30         to the range of about pH 6.8 to about pH 6.0, to the range of about pH 6.8 to about pH 6.5, or to the range of about pH 6.5 to about pH 6.0. In some embodiments, the pH of the cell lysate or sample can be reduced to any range having any combination of endpoints from

about pH 6.0 to about pH 9.0  All such endpoints and ranges are specifically and separately contemplated.

In some embodiments, the cells are not lysed by heat.  Those of skill in the art will understand that different cells under different conditions will be lysed at different

5       temperatures and so can determine temperatures and times at which the cells will not be lysed by heat.  In general, the cells are not subjected to heating above a temperature and for a time that would cause substantial cell lysis in the absence of the alkaline conditions used. As used herein, substantial cell lysis refers to lysis of 90% or greater of the cells exposed to the alkaline conditions.  Significant cell lysis refers to lysis of 50% or more of the cells

10      exposed to the alkaline conditions.  Sufficient cell lysis refers to lysis of enough of the cells exposed to the alkaline conditions to allow synthesis of a detectable amount of amplification products by multiple strand displacement amplification.  In general, the alkaline conditions used in the disclosed method need only cause sufficient cell lysis.  It should be understood that alkaline conditions that could cause significant or substantial cell lysis need not result

15      in significant or substantial cell lysis when the method is performed.

In some embodiments, the cells are not subjected to heating substantially or significantly above the temperature at which the cells grow.  As used herein, the temperature at which the cells grow refers to the standard temperature, or highest of different standard temperatures, at which cells of the type involved are cultured.  In the case

20      of animal cells, the temperature at which the cells grow refers to the body temperature of the animal.  In other embodiments, the cells are not subjected to heating substantially or significantly above the temperature of the amplification reaction (where the genome is replicated).

In some embodiments, the cell lysate or sample is not subjected to purification prior

25      to the amplification reaction.  In the context of the disclosed method, purification generally refers to the separation of nucleic acids from other material in the cell lysate or sample.  It has been discovered that multiple displacement amplification can be performed on unpurified and partially purified samples.  It is commonly thought that amplification reactions cannot be efficiently performed using unpurified nucleic acid.  In particular, PCR

30      is very sensitive to contaminants.

Forms of purification include centrifugation, extraction, chromatography, precipitation, filtration, and dialysis. Partially purified cell lysate or samples includes cell lysates or samples subjected to centrifugation, extraction, chromatography, precipitation,

106

filtration, and dialysis. Partially purified cell lysate or samples generally does not include cell lysates or samples subjected to nucleic acid precipitation or dialysis. As used herein, separation of nucleic acid from other material refers to physical separation such that the nucleic acid to be amplified is in a different container or container from the material.

5      Purification does not require separation of all nucleic acid from all other materials. Rather, what is required is separation of some nucleic acid from some other material. As used herein in the context of nucleic acids to be amplified, purification refers to separation of nucleic acid from other material. In the context of cell lysates, purification refers to separation of nucleic acid from other material in the cell lysate. As used herein, partial

10     purification refers to separation of nucleic acid from some, but not all, of other material with which the nucleic acid is mixed. In the context of cell lysates, partial purification refers to separation of nucleic acid from some, but not all, of the other material in the cell lysate.

Unless the context clearly indicates otherwise, reference herein to a lack of purification, lack of one or more types of purification or separation operations or

15     techniques, or exclusion of purification or one or more types of purification or separation operations or techniques does not encompass the exposure of cells or samples to alkaline conditions (or the results thereof) or the reduction of pH of a cell lysate or sample (or the results thereof). That is, to the extent that the alkaline conditions and pH reduction of the disclosed method produce an effect that could be considered "purification" or "separation,"

20     such effects are excluded from the definition of purification and separation when those terms are used in the context of processing and manipulation of cell lysates, samples, stabilized samples and stabilized cell lysates (unless the context clearly indicates otherwise).

As used herein, substantial purification refers to separation of nucleic acid from at least a substantial portion of other material with which the nucleic acid is mixed. In the

25     context of cell lysates, substantial purification refers to separation of nucleic acid from at least a substantial portion of the other material in the cell lysate. A substantial portion refers to 90% of the other material involved. Specific levels of purification can be referred to as a percent purification (such as 95% purification and 70% purification). A percent purification refers to purification that results in separation from nucleic acid of at least the designated

30     percent of other material with which the nucleic acid is mixed.

Denaturation of nucleic acid molecules to be amplified is common in amplification techniques. This is especially true when amplifying genomic DNA. In particular, PCR uses multiple denaturation cycles. Denaturation is generally used to make nucleic acid strands

107

accessible to primers. It was discovered that the target nucleic acids, genomic DNA, for example, need not be denatured for efficient multiple displacement amplification. It was also discovered that elimination of a denaturation step and denaturation conditions has additional advantages such as reducing sequence bias in the amplified products. In some

5      embodiments, the nucleic acids in the cell lysate or sample are not denatured by heating. In some embodiments, the cell lysate is not subjected to heating substantially or significantly above the temperature at which the cells grow. In other embodiments, the cell lysate or sample is not subjected to heating substantially or significantly above the temperature of the amplification reaction (where the genome is replicated). The disclosed multiple

10     displacement amplification reaction is generally conducted at a substantially constant temperature (that is, the amplification reaction is substantially isothermic), and this temperature is generally below the temperature at which the nucleic acids would be notably denatured. As used herein, notable denaturation refers to denaturation of 10% or greater of the base pairs.

15           In preferred forms of the disclosed method, the nucleic acid sample or template nucleic acid is not subjected to denaturing conditions and/or no denaturation step is used. In some forms of the disclosed method, the nucleic acid sample or template nucleic acid is not subjected to heat denaturing conditions and/or no heat denaturation step is used. It should be understood that while sample preparation (for example, cell lysis and processing of cell

20     extracts) may involve conditions that might be considered denaturing (for example, treatment with alkali), the denaturation conditions or step eliminated in some forms of the disclosed method refers to denaturation steps or conditions intended and used to make nucleic acid strands accessible to primers. Such denaturation is commonly a heat denaturation, but can also be other forms of denaturation such as chemical denaturation. It

25     should be understood that in the disclosed method where the nucleic acid sample or template nucleic acid is not subjected to denaturing conditions, the template strands are accessible to the primers (since amplification occurs). However, the template stands are not made accessible via general denaturation of the sample or template nucleic acids.

            The pH of all or a portion of a sample or cells exposed to alkaline conditions can be

30     reduced to form a stabilized or neutralized sample or cell lysate, and an amplification mixture can comprise all or a portion of the neutralized or stabilized sample or cell lysate. An amplification mixture is the reaction solution where nucleic acids are amplified. An amplification mixture can comprise a genome, and the genome can comprise any fraction of

the nucleic acids in the amplification mixture. The genome can comprise, for example, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at

5      least 98%, or at least 99% of the nucleic acids in the amplification mixture.

The concentration of nucleic acids in an amplification mixture can favor hybridization of primers over reassociation of the nucleic acids, which serves to improve the quality of the amplification products (by, for example, providing a lower amplification bias). The concentration at or below which low amplification bias can be achieved can be

10     determined for different samples and for different amplification techniques using methods described herein. The concentration of nucleic acids in the amplification mixture can be, for example, 300 ng/µl or less, 200 ng/µl or less, 150 ng/µl or less, 100 ng/µl or less, 95 ng/µl or less, 90 ng/µl or less, 85 ng/µl or less, 80 ng/µl or less, 75 ng/µl or less, 70 ng/µl or less, 65 ng/µl or less, 60 ng/µl or less, 55 ng/µl or less, 50 ng/µl or less, 45 ng/µl or less, 40

15     ng/µl or less, 35 ng/µl or less, 30 ng/µl or less, 25 ng/µl or less, 20 ng/µl or less, 15 ng/µl or less, 10 ng/µl or less, 9 ng/µl or less, 8 ng/µl or less, 7 ng/µl or less, 6 ng/µl or less, 5 ng/µl or less, 4 ng/µl or less, 3 ng/µl or less, 2 ng/µl or less, 1 ng/µl or less, 0.8 ng/µl or less, 0.6 ng/µl or less, 0.5 ng/µl or less, 0.4 ng/µl or less, 0.3 ng/µl or less, 0.2 ng/µl or less, or 0.1 ng/µl or less.

20     The amount of nucleic acids in an amplification mixture can be at or above a threshold amount, which serves to improve the quality of the amplification products (by, for example, providing a lower amplification bias). The amount at or above which low amplification bias can be achieved can be determined for different samples and for different amplification techniques using methods described herein. The amount of nucleic acids in

25     the amplification mixture can be, for example, at least 50 ng, at least 60 ng, at least 70 ng, at least 80 ng, at least 90 ng, at least 100 ng, at least 110 ng, at least 120 ng, at least 130 ng, at least 140 ng, at least 150 ng, at least 160 ng, at least 170 ng, at least 180 ng, at least 190 ng, at least 200 ng, at least 220 ng, at least 240 ng, at least 260 ng, at least 280 ng, at least 300 ng, at least 325 ng, at least 350 ng, at least 375 ng, at least 400 ng, at least 450 ng, or at least

30     500 ng.

The efficiency of a DNA amplification procedure may be described for individual loci as the percent representation, where the percent representation is 100% for a locus in genomic DNA as purified from cells. For 10,000-fold amplification, the average

representation frequency was 141% for 8 loci in DNA amplified without heat denaturation of the template, and 37% for the 8 loci in DNA amplified with heat denaturation of the template. The omission of a heat denaturation step results in a 3.8-fold increase in the representation frequency for amplified loci. Amplification bias may be calculated between two samples of amplified DNA or between a sample of amplified DNA and the template DNA it was amplified from. The bias is the ratio between the values for percent representation (or for locus representation) for a particular locus. The maximum bias is the ratio of the most highly represented locus to the least represented locus. For 10,000-fold amplification, the maximum amplification bias was 2.8 for DNA amplified without heat denaturation of the template, and 50.7 for DNA amplified with heat denaturation of the template. The omission of a heat denaturation step results in an 18-fold decrease in the maximum bias for amplified loci. Percent representation is a form of representation bias. Thus, percent locus representation is a form of locus representation bias.

The disclosed methods can produce high quality amplification products. For example, the disclosed methods can produce a locus representation or locus representation bias of at least 10% for at least 5 different loci, a sequence representation or sequence representation bias of at least 10% for at least 5 different target sequences, an amplification bias of less than 50-fold, an amplification bias of less than 50-fold for at least 5 different loci, and/or an amplification bias of less than 50-fold for at least 5 different target sequences. The disclosed methods can also produce, for example, a locus representation or locus representation bias of at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% for at least 5 different loci. The disclosed methods can also produce, for example, a locus representation or locus representation bias of at least 10% for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

The disclosed methods can also produce, for example, a sequence representation or sequence representation bias of at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%,

at least 90%, or at least 100% for at least 5 different target sequences. Some forms of the disclosed methods can also produce, for example, a sequence representation or sequence representation bias of at least 10% for at least 6 different target sequences, at least 7 different target sequences, at least 8 different target sequences, at least 9 different target

5    sequences, at least 10 different target sequences, at least 11 different target sequences, at least 12 different target sequences, at least 13 different target sequences, at least 14 different target sequences, at least 15 different target sequences, at least 16 different target sequences, at least 17 different target sequences, at least 18 different target sequences, at least 19 different target sequences, at least 20 different target sequences, at least 25 different target

10   sequences, at least 30 different target sequences, at least 40 different target sequences, at least 50 different target sequences, at least 75 different target sequences, or at least 100 different target sequences.

The disclosed methods can also produce, for example, an amplification bias of less than 45-fold, less than 40-fold, less than 35-fold, less than 30-fold, less than 25-fold, less

15   than 20-fold, less than 19-fold, less than 18-fold, less than 17-fold, less than 16-fold, less than 15-fold, less than 14-fold, less than 13-fold, less than 12-fold, less than 11-fold, less than 10-fold, less than 9-fold, less than 8-fold, less than 7-fold, less than 6-fold, less than 5-fold, or less than 4-fold. The disclosed methods can also produce, for example, an amplification bias of less than 50-fold for at least 5 different loci, for at least 6 different loci,

20   at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75

25   different loci, or at least 100 different loci. The disclosed methods can also produce, for example, an amplification bias of less than 50-fold for at least 5 different target sequences, for at least 6 different target sequences, at least 7 different target sequences, at least 8 different target sequences, at least 9 different target sequences, at least 10 different target sequences, at least 11 different target sequences, at least 12 different target sequences, at

30   least 13 different target sequences, at least 14 different target sequences, at least 15 different target sequences, at least 16 different target sequences, at least 17 different target sequences, at least 18 different target sequences, at least 19 different target sequences, at least 20 different target sequences, at least 25 different target sequences, at least 30 different target

sequences, at least 40 different target sequences, at least 50 different target sequences, at least 75 different target sequences, or at least 100 different target sequences. These results can be over a variety of samples, for some selected types of samples, or for a specific type of sample.

As used herein, a low amplification bias includes amplification biases of less than 10-fold for at least 5 sequences or loci, less than 12-fold for at least 6 sequences or loci, less than 14-fold for at least 7 sequences or loci, less than 16-fold for at least 8 sequences or loci, less than 18-fold for at least 9 sequences or loci, less than 20-fold for at least 10 sequences or loci, less than 22-fold for at least 11 sequences or loci, less than 24-fold for at least 12 sequences or loci, less than 26-fold for at least 13 sequences or loci, less than 28-fold for at least 14 sequences or loci, less than 30-fold for at least 15 sequences or loci, less than 32-fold for at least 16 sequences or loci, less than 34-fold for at least 17 sequences or loci, less than 36-fold for at least 18 sequences or loci, less than 38-fold for at least 19 sequences or loci, less than 40-fold for at least 20 sequences or loci, less than 42-fold for at least 21 sequences or loci, less than 44-fold for at least 22 sequences or loci, less than 46-fold for at least 23 sequences or loci, less than 48-fold for at least 24 sequences or loci, and less than 50-fold for at least 25 sequences or loci. Generalizing, low amplification bias includes amplification biases of 2x-fold where x is the number of sequences or loci over which the amplification bias is calculated or observed. Low amplification bias can be expressed in other ways, such as by allele bias, locus representation, sequence representation, allele representation, locus representation bias, sequence representation bias, percent representation, percent locus representation, percent sequence representation, and other measures that indicate low bias and/or complete amplification of the input nucleic acids. The values of such other measures that constitute low amplification bias generally can be calculated by reference to the above definition and formula in view of the relationships between amplification bias and other measures of bias described elsewhere herein.

In another form of the method, the primers can be hexamer primers. It was discovered that such short, 6 nucleotide primers can still prime multiple strand displacement replication efficiently. Such short primers are easier to produce as a complete set of primers of random sequence (random primers) than longer primers at least because there are fewer to make. In another form of the method, the primers can each contain at least one modified nucleotide such that the primers are nuclease resistant. In another form of the method, the

112

primers can each contain at least one modified nucleotide such that the melting temperature of the primer is altered relative to a primer of the same sequence without the modified nucleotide(s). In another form of the method, the DNA polymerase can be φ29 DNA polymerase. It was discovered that φ29 DNA polymerase produces greater amplification in

5    multiple displacement amplification. The combination of two or more of the above features also yields improved results in multiple displacement amplification. In a preferred embodiment, for example, the target sample is not subjected to denaturing conditions, the primers are hexamer primers and contain modified nucleotides such that the primers are nuclease resistant, and the DNA polymerase is φ29 DNA polymerase. The above features

10   are especially useful in whole genome strand displacement amplification (WGSDA).

In another form of the disclosed method, the method includes labeling of the replicated strands (that is, the strands produced in multiple displacement amplification) using terminal deoxynucleotidyl transferase. The replicated strands can be labeled by, for example, the addition of modified nucleotides, such as biotinylated nucleotides, fluorescent

15   nucleotides, 5 methyl dCTP, BrdUTP, or 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphates, to the 3' ends of the replicated strands.

Some forms of the disclosed method provide amplified DNA of higher quality relative to previous methods due to the lack of a heat denaturation treatment of the DNA that is the target for amplification. Thus, the template DNA does not undergo the strand

20   breakage events caused by heat treatment and the amplification that is accomplished by a single DNA polymerase extends farther along template strands of increased length.

In one form of the disclosed method, a small amount of purified double-strand human genomic DNA (1 ng, for example) can be mixed with exonuclease-resistant random hexamer primers and φ29 DNA polymerase under conditions that favor DNA synthesis. For

25   example, the mixture can simply be incubated at 30°C and multiple displacement amplification will take place. Thus, any single-stranded or duplex DNA may be used, without any additional treatment, making the disclosed method a simple, one-step procedure. Since so little DNA template is required, a major advantage of the disclosed method is that DNA template may be taken from preparations that contain levels of

30   contaminants that would inhibit other DNA amplification procedures such as PCR. For MDA the sample may be diluted so that the contaminants fall below the concentration at which they would interfere with the reaction. The disclosed method can be performed (and

the above advantages achieved) using any type of sample, including, for example, bodily fluids such as urine, semen, lymphatic fluid, cerebrospinal fluid, and amniotic fluid.

The need for only small amounts of DNA template in the disclosed method means that the method is useful for DNA amplification from very small samples. In particular, the disclosed method may be used to amplify DNA from a single cell. The ability to obtain analyzable amounts of nucleic acid from a single cell (or similarly small sample) has many applications in preparative, analytical, and diagnostic procedures such as prenatal diagnostics. Other examples of biological samples containing only small amounts of DNA for which amplification by the disclosed method would be useful are material excised from tumors or other archived medical samples, needle aspiration biopsies, clinical samples arising from infections, such as nosocomial infections, forensic samples, or museum specimens of extinct species.

More broadly, the disclosed method is useful for applications in which the amounts of DNA needed are greater than the supply. For example, procedures that analyze DNA by chip hybridization techniques are limited by the amounts of DNA that can be purified from typically sized blood samples. As a result many chip hybridization procedures utilize PCR to generate a sufficient supply of material for the high-throughput procedures. The disclosed method presents a useful technique for the generation of plentiful amounts of amplified DNA that faithfully reproduces the locus representation frequencies of the starting material.

Whole genome amplification by MDA can be carried out directly from blood or cells bypassing the need to isolate pure DNA. For example, blood or other cells can be lysed by dilution with an equal volume of 2X Alkaline Lysis Buffer (400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA), an example of a lysis solution, and incubated 10 minutes on ice. The lysed cells can be stabilized or neutralized with the same volume of Neutralization Buffer (800 mM Tris-HCl, pH 4.1), an example of a stabilization solution. Preparations of lysed blood or cells (for example, 1 ml) can used directly as template in MDA reactions (for example, 100 ml). If desired, prior to lysis, blood can be diluted 3-fold in phosphate buffered saline (PBS) and tissue culture cells can be diluted to 30,000 cells/ml in PBS.

It has been discovered that it is unnecessary to have prior knowledge of whether or not a sample contains amplifiable nucleic acids. Some forms of the disclosed methods can be employed to test whether or not a sample suspected of containing nucleic acids actually

114

does contain nucleic acids. Production of amplified DNA from such samples using the disclosed method is evidence that the sample contained nucleic acids. More generally, practice of the disclosed methods does not require any knowledge of any nucleic acid sequence in a sample. Thus, the disclosed methods can be used to amplify nucleic acids

5 from any source, regardless of a lack of specific sequence information. This is in contrast to other amplification methods, such as PCR, where it is necessary to have prior information of at least a portion of the nucleic acid sequences believed to be present in the sample in order to perform the amplification. In this instance, the PCR amplification reaction will fail if the nucleic acids present in the sample are different from the expected sample nucleic

10 acids. If a sample contains a mixture of nucleic acids, then nucleic acids of the appropriate type alone will be amplified in a PCR reaction, but not the other types of nucleic acids. In contrast, the disclosed methods provide for amplification of most or all of the nucleic acids present in the sample. The disclosed methods are equally adaptable to using samples that conventionally are not expected or believed to contain nucleic acids. For instance, serum or

15 plasma from humans or other higher animals were believed to not contain free host nucleic acids. However, it was discovered that the disclosed methods could amplify nucleic acids present in such samples.

A form of the disclosed method can be illustrated by the following protocol. This protocol can be used for any type of sample, such as cell samples and nucleic acid samples.

20 1. Denaturation of the genomic DNA template before amplification. Prepare the Lysis Solution by diluting Solution A by 1:4 with $H_2O$ (e.g. 100 µL of Solution A into 300 µL of $H_2O$). Prepare the Stabilization Buffer by diluting Solution B by 1:5 with $H_2O$ (for example, 100 µL of Solution A into 400 µL of $H_2O$). Both Lysis and Stabilization Solution should be prepared fresh before each new experiment. After use, the bottle containing

25 Solution A should be resealed immediately to avoid neutralization from $CO_2$.

Solution A has a useful shelf-life of 6 months. Prepare a fresh Solution A if it has been stored more than 6 months.

Solution A:     400 mM KOH, 10 mM EDTA, pH 8

Solution B:     800 mM Tris Hydrochloride, pH 4

30 2. Add 2.5 µL of the Lysis Solution to each 0.2 mL thermocycler tube containing 2.5 µL of genomic DNA on ice. Mix well by pipetting up and down 5 times. Incubate the tubes or plate on ice for 3 minutes.

115

3. Stop the denaturation reaction after 3 minutes by adding 5 µL of the Stabilization Buffer to each sample and control. Remove the tubes from ice. Proceed immediately to the amplification reaction.

4. To the tube from Step 3, add in a final volume of 50 µl:

5

Required amount of genomic DNA,

0.3M Trehalose,

10 mM MgCl$_2$,

37.5 mM Tris/HCl pH: 7,

50 mM KCl,

10

20 mM Ammonium Sulfate,

1 mM dNTPs,

50 µM exonuclease-resistant random hexamer oligonucleotide,

40 units of Phi29 DNA polymerase.

Incubate at 40 °C for 6-16 hrs.

15

A specific embodiment of the disclosed method is described in Example 4, wherein whole genome amplification is performed by MDA without heat treatment of the human template DNA. As shown in the example, the disclosed method produces a DNA amplification product with improved performance in genetic assays compared to

20    amplification performed with heat treatment of the template DNA. The longer DNA products produced without heat treatment of the template yield larger DNA fragments in Southern blotting and genetic analysis using RFLP.

The breakage of DNA strands by heat treatment is demonstrated directly in Example 5, while the decreased rate and yield of DNA amplification from heat-treated DNA is

25    depicted in Example 6. The decrease in DNA product strand length resulting from heat treatment of the DNA template is demonstrated in Example 7.

A specific form of the disclosed method is described in Example 8, wherein purified human genomic DNA is amplified by MDA without heat treatment of the template. As shown in the example, the disclosed method produces for a DNA amplification product with

30    no loss of locus representation when used as a substrate in quantitative PCR assays compared to DNA amplified with heat treatment of the template.

Another specific form of the disclosed method is described in Example 9, wherein purified human genomic DNA is amplified by MDA without heat treatment of the template.

As shown in the example, the disclosed method produces a DNA amplification product with a low amplification bias, with the variation in representation among eight different loci varying by less than 3.0. In contrast, the amplification bias of DNA products amplified by two PCR-based amplification methods, PEP and DOP-PCR, varies between two and six

5      orders of magnitude.

Another specific form of the disclosed method is described in Example 10, wherein the amplification of c-jun sequences using specific, nested primers from a human genomic DNA template is enhanced by omission of a DNA template heat denaturation step.

Another specific form of the disclosed method is described in Example 11, wherein

10     human genomic DNA is amplified in the absence of a heat treatment step directly from whole blood or from tissue culture cells with the same efficiency as from purified DNA. The DNA amplified directly from blood or cells has substantially the same locus representation values as DNA amplified from purified human DNA template. This represents an advantage over other amplification procedures such as PCR, since

15     components such as heme in whole blood inhibit PCR and necessitate a purification step before DNA from blood can be used as a PCR template.

Another specific form of the disclosed method is described in Example 12, wherein purified human genomic DNA is amplified by MDA without heat treatment of the template in the presence of 70% AA-dUTP / 30% dTTP. As shown in the example, the disclosed

20     method provides for a DNA amplification product with the same low amplification bias as for DNA amplified in the presence of 100% dTTP.

Also disclosed is a method for amplifying and repairing damaged DNA. This method is useful, for example, for amplifying degraded genomic DNA. The method involves substantially denaturing a damaged DNA sample (generally via exposure to heat

25     and alkaline conditions), removal or reduction of the denaturing conditions (such as by reduction of the pH and temperature of the denatured DNA sample), and replicating the DNA. The damaged DNA is repaired during replication and the average length of DNA fragments is increased. For example, the average length of DNA fragments can be increase from, for example, 2 kb in the damaged DNA sample to, for example, 10 kb or greater for

30     the replicated DNA. The amplified and repaired DNA is in better condition for analysis and testing than the damaged DNA sample. For example, this technique can provide consistent improvements in allele representation from damaged DNA samples. This repair method can result in an overall improvement in amplification of damaged DNA by increasing the

average length of the product, increasing the quality of the amplification products by 3-fold (by, for example, increasing the marker representation in the sample), and improving the genotyping of amplified products by lowering the frequency of allelic dropout; all compared to the results when amplifying damaged DNA by other methods. The replication can be multiple displacement amplification. Denaturation of the DNA sample generally is carried out such that the DNA is not further damaged. This method can generally be combined or used with any of the disclosed amplification methods. Another form of this method can involve substantially denaturing a damaged DNA sample (generally via exposure to heat and alkaline conditions), reduction of the pH of the denatured DNA sample, mixing the denatured DNA sample with an undenatured DNA sample from the same source such that the ends of DNA in the undenatured DNA sample is transiently denatured, slowly cooling the mixture of DNA samples to allow the transiently denatured ends to anneal to the denatured DNA, and replicating the annealed DNA.

The disclosed methods, either in whole or in part, can be performed in or on solid supports or in or on reaction chambers. For example, the disclosed replication, incubation and amplification steps can be performed with the amplification mixture in or on solid supports or in or on reaction chambers. For example, the disclosed replication, incubation and amplification steps can be performed with the amplification mixture on solid supports having reaction chambers. A reaction chamber is any structure in which a separate amplification reaction can be performed. Useful reaction chambers include tubes, test tubes, eppendorf tubes, vessels, micro vessels, plates, wells, wells of micro well plates, wells of microtitre plates, chambers, micro fluidics chambers, micro machined chambers, sealed chambers, holes, depressions, dimples, dishes, surfaces, membranes, microarrays, fibers, glass fibers, optical fibers, woven fibers, films, beads, bottles, chips, compact disks, shaped polymers, particles, microparticles or other structures that can support separate reactions. Reaction chambers can be made from any suitable material. Such materials include acrylamide, cellulose, nitrocellulose, glass, gold, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, glass, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, functionalized silane, polypropylfumerate, collagen, glycosaminoglycans, and polyamino acids. Solid supports preferably comprise arrays of reaction chambers. In connection with reaction chambers, a separate reaction refers to a reaction where substantially no cross contamination of reactants

118

or products will occur between different reaction chambers. Substantially no cross contamination refers to a level of contamination of reactants or products below a level that would be detected in the particular reaction or assay involved. For example, if nucleic acid contamination from another reaction chamber would not be detected in a given reaction

5      chamber in a given assay (even though it may be present), there is no substantial cross contamination of the nucleic acid. It is understood, therefore, that reaction chambers can comprise, for example, locations on a planar surface, such as spots, so long as the reactions performed at the locations remain separate and are not subject to mixing.

Some forms of the disclosed method are based on strand displacement replication of

10     the nucleic acid sequences by one, a few, or more primers. The method can be used to amplify an nucleic acid sample and is particularly useful for amplifying nucleic acid samples having a high sequence complexity, such as entire genomes. The disclosed method can be used to amplify such highly complex nucleic acid samples using only one or a limited number of primers. It has been discovered that one or a small number of primers

15     can effectively amplify whole genomes and other nucleic acid samples of high sequence complexity. The primers are specially selected or designed to be able to prime and efficiently amplify the broad range of sequences present in highly complex nucleic acid samples despite the limited amount of primer sequence represented in the primers. The disclosed method generally involves bringing into contact one, a few, or more primers

20     having specific nucleic acid sequences, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under conditions that promote replication of nucleic acid molecules in the nucleic acid sample. Replication of the nucleic acid molecules results in replicated strands such that, during replication, the replicated strands are displaced from the nucleic acid molecules by strand displacement replication of another replicated strand. The

25     replication can result in amplification of all or a substantial fraction of the nucleic acid molecules in the nucleic acid sample. As used herein, a replicated strand is a nucleic acid strand resulting from elongation of a primer hybridized to a nucleic acid molecule or nucleic acid sequence or to another replicated strand. Strand displacement replication refers to DNA replication where a growing end of a replicated strand encounters and displaces

30     another strand from the template strand (or from another replicated strand). Displacement of replicated strands by other replicated strands is a hallmark of the disclosed method which allows multiple copies of nucleic acid molecules or nucleic acid sequences to be made in a single, isothermic reaction.

In another form of the method, the primers can be 6 nucleotides in length. It was discovered that such short, 6 nucleotide primers can still prime multiple strand displacement replication efficiently. In another form of the method, the primers can each contain at least one modified nucleotide such that the primers are nuclease resistant. In another form of the

5    method, the primers can each contain at least one modified nucleotide such that the melting temperature of the primer is altered relative to a primer of the same sequence without the modified nucleotide(s). In another form of the method, the DNA polymerase can be φ29 DNA polymerase. φ29 DNA polymerase produces greater amplification in multiple displacement amplification. The combination of two or more of the above features also

10   yields improved results in multiple displacement amplification. In a preferred embodiment, for example, the nucleic acid sample is not subjected to denaturing conditions, the primers are 6 nucleotides long and contain modified nucleotides such that the primers are nuclease resistant, and the DNA polymerase is φ29 DNA polymerase. The above features are especially useful in whole genome strand displacement amplification (WGSDA).

15       In another form of the disclosed method, the method includes labeling of the replicated strands (that is, the strands produced in multiple displacement amplification) using terminal deoxynucleotidyl transferase. The replicated strands can be labeled by, for example, the addition of modified nucleotides, such as biotinylated nucleotides, fluorescent nucleotides, 5 methyl dCTP, BrdUTP, or 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphates,

20   to the 3' ends of the replicated strands.

Some forms of the disclosed method provide amplified DNA of higher quality relative to previous methods due to the lack of a heat denaturation treatment of the nucleic acid molecules that are the target for amplification. Thus, the template DNA does not undergo the strand breakage events caused by heat treatment and the amplification that is

25   accomplished by a single DNA polymerase extends farther along template strands of increased length.

## A. Amplification Level

The disclosed method can produce a high level of amplification. For example, the disclosed method can produce a 10,000-fold amplification or more. Fold amplification

30   refers to the number of copies generated of the template being amplified. For example, if 1 ug of DNA is generated from 1 ng of template, the level of amplification is 1,000-fold. The disclosed method can produce, for example, amplification of about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-

fold, about 10-fold, about 11-fold, about 12-fold, about 14-fold, about 16-fold, about 20-fold, about 24-fold, about 30-fold, about 35-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, about 100-fold, about 150-fold, about 200-fold, about 250-fold, about 300-fold, about 400-fold, about 500-fold, about 600-fold, about

5       700-fold, about 800-fold, about 900-fold, about 1,000-fold, about 10,000-fold, about 100,000-fold, about 1,000,000-fold, about 10,000,000-fold, or about 100,000,000-fold.

The disclosed method can produce, for example, amplification of at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 11-fold, at least 12-fold, at least 14-fold, at least 16-

10      fold, at least 20-fold, at least 24-fold, at least 30-fold, at least 35-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, at least 100-fold, at least 150-fold, at least 200-fold, at least 250-fold, at least 300-fold, at least 400-fold, at least 500-fold, at least 600-fold, at least 700-fold, at least 800-fold, at least 900-fold, at least 1,000-fold, at least 10,000-fold, at least 100,000-fold, at least 1,000,000-fold, at least

15      10,000,000-fold, or at least 100,000,000-fold.

The disclosed method can produce, for example, amplification bias of at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 11-fold, at least about 12-fold, at least about 14-fold, at least about 16-fold, at

20      least about 20-fold, at least about 24-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 50-fold, at least about 60-fold, at least about 70-fold, at least about 80-fold, at least about 90-fold, at least about 100-fold, at least about 150-fold, at least about 200-fold, at least about 250-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-

25      fold, at least about 900-fold, at least about 1,000-fold, at least about 10,000-fold, at least about 100,000-fold, at least about 1,000,000-fold, at least about 10,000,000-fold, or at least about 100,000,000-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 100,000,000-fold, from about 2-fold to about 100,000,000-fold, from about 3-fold

30      to about 100,000,000-fold, from about 4-fold to about 100,000,000-fold, from about 5-fold to about 100,000,000-fold, from about 6-fold to about 100,000,000-fold, from about 7-fold to about 100,000,000-fold, from about 8-fold to about 100,000,000-fold, from about 9-fold to about 100,000,000-fold, from about 10-fold to about 100,000,000-fold, from about 11-

121

fold to about 100,000,000-fold, from about 12-fold to about 100,000,000-fold, from about 14-fold to about 100,000,000-fold, from about 16-fold to about 100,000,000-fold, from about 20-fold to about 100,000,000-fold, from about 24-fold to about 100,000,000-fold, from about 30-fold to about 100,000,000-fold, from about 35-fold to about 100,000,000-fold, from about 40-fold to about 100,000,000-fold, from about 50-fold to about 100,000,000-fold, from about 60-fold to about 100,000,000-fold, from about 70-fold to about 100,000,000-fold, from about 80-fold to about 100,000,000-fold, from about 90-fold to about 100,000,000-fold, from about 100-fold to about 100,000,000-fold, from about 150-fold to about 100,000,000-fold, from about 200-fold to about 100,000,000-fold, from about 250-fold to about 100,000,000-fold, from about 300-fold to about 100,000,000-fold, from about 400-fold to about 100,000,000-fold, from about 500-fold to about 100,000,000-fold, from about 600-fold to about 100,000,000-fold, from about 700-fold to about 100,000,000-fold, from about 800-fold to about 100,000,000-fold, from about 900-fold to about 100,000,000-fold, from about 1,000-fold to about 100,000,000-fold, from about 10,000-fold to about 100,000,000-fold, from about 100,000-fold to about 100,000,000-fold, from about 1,000,000-fold to about 100,000,000-fold, or from about 10,000,000-fold to about 100,000,000-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 10,000,000-fold, from about 2-fold to about 10,000,000-fold, from about 3-fold to about 10,000,000-fold, from about 4-fold to about 10,000,000-fold, from about 5-fold to about 10,000,000-fold, from about 6-fold to about 10,000,000-fold, from about 7-fold to about 10,000,000-fold, from about 8-fold to about 10,000,000-fold, from about 9-fold to about 10,000,000-fold, from about 10-fold to about 10,000,000-fold, from about 11-fold to about 10,000,000-fold, from about 12-fold to about 10,000,000-fold, from about 14-fold to about 10,000,000-fold, from about 16-fold to about 10,000,000-fold, from about 20-fold to about 10,000,000-fold, from about 24-fold to about 10,000,000-fold, from about 30-fold to about 10,000,000-fold, from about 35-fold to about 10,000,000-fold, from about 40-fold to about 10,000,000-fold, from about 50-fold to about 10,000,000-fold, from about 60-fold to about 10,000,000-fold, from about 70-fold to about 10,000,000-fold, from about 80-fold to about 10,000,000-fold, from about 90-fold to about 10,000,000-fold, from about 100-fold to about 10,000,000-fold, from about 150-fold to about 10,000,000-fold, from about 200-fold to about 10,000,000-fold, from about 250-fold to about 10,000,000-fold, from about 300-fold to about 10,000,000-fold, from about 400-fold to about 10,000,000-fold, from about

500-fold to about 10,000,000-fold, from about 600-fold to about 10,000,000-fold, from about 700-fold to about 10,000,000-fold, from about 800-fold to about 10,000,000-fold, from about 900-fold to about 10,000,000-fold, from about 1,000-fold to about 10,000,000-fold, from about 10,000-fold to about 10,000,000-fold, from about 100,000-fold to about 10,000,000-fold, or from about 1,000,000-fold to about 10,000,000-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 1,000,000-fold, from about 2-fold to about 1,000,000-fold, from about 3-fold to about 1,000,000-fold, from about 4-fold to about 1,000,000-fold, from about 5-fold to about 1,000,000-fold, from about 6-fold to about 1,000,000-fold, from about 7-fold to about 1,000,000-fold, from about 8-fold to about 1,000,000-fold, from about 9-fold to about 1,000,000-fold, from about 10-fold to about 1,000,000-fold, from about 11-fold to about 1,000,000-fold, from about 12-fold to about 1,000,000-fold, from about 14-fold to about 1,000,000-fold, from about 16-fold to about 1,000,000-fold, from about 20-fold to about 1,000,000-fold, from about 24-fold to about 1,000,000-fold, from about 30-fold to about 1,000,000-fold, from about 35-fold to about 1,000,000-fold, from about 40-fold to about 1,000,000-fold, from about 50-fold to about 1,000,000-fold, from about 60-fold to about 1,000,000-fold, from about 70-fold to about 1,000,000-fold, from about 80-fold to about 1,000,000-fold, from about 90-fold to about 1,000,000-fold, from about 100-fold to about 1,000,000-fold, from about 150-fold to about 1,000,000-fold, from about 200-fold to about 1,000,000-fold, from about 250-fold to about 1,000,000-fold, from about 300-fold to about 1,000,000-fold, from about 400-fold to about 1,000,000-fold, from about 500-fold to about 1,000,000-fold, from about 600-fold to about 1,000,000-fold, from about 700-fold to about 1,000,000-fold, from about 800-fold to about 1,000,000-fold, from about 900-fold to about 1,000,000-fold, from about 1,000-fold to about 1,000,000-fold, from about 10,000-fold to about 1,000,000-fold, or from about 100,000-fold to about 1,000,000-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 100,000-fold, from about 2-fold to about 100,000-fold, from about 3-fold to about 100,000-fold, from about 4-fold to about 100,000-fold, from about 5-fold to about 100,000-fold, from about 6-fold to about 100,000-fold, from about 7-fold to about 100,000-fold, from about 8-fold to about 100,000-fold, from about 9-fold to about 100,000-fold, from about 10-fold to about 100,000-fold, from about 11-fold to about 100,000-fold, from about 12-fold to about 100,000-fold, from about 14-fold to about 100,000-fold, from about 16-fold to about 100,000-fold, from about 20-fold to about 100,000-fold, from about 24-fold to

about 100,000-fold, from about 30-fold to about 100,000-fold, from about 35-fold to about 100,000-fold, from about 40-fold to about 100,000-fold, from about 50-fold to about 100,000-fold, from about 60-fold to about 100,000-fold, from about 70-fold to about 100,000-fold, from about 80-fold to about 100,000-fold, from about 90-fold to about 100,000-fold, from about 100-fold to about 100,000-fold, from about 150-fold to about 100,000-fold, from about 200-fold to about 100,000-fold, from about 250-fold to about 100,000-fold, from about 300-fold to about 100,000-fold, from about 400-fold to about 100,000-fold, from about 500-fold to about 100,000-fold, from about 600-fold to about 100,000-fold, from about 700-fold to about 100,000-fold, from about 800-fold to about 100,000-fold, from about 900-fold to about 100,000-fold, from about 1,000-fold to about 100,000-fold, or from about 10,000-fold to about 100,000-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 10,000-fold, from about 2-fold to about 10,000-fold, from about 3-fold to about 10,000-fold, from about 4-fold to about 10,000-fold, from about 5-fold to about 10,000-fold, from about 6-fold to about 10,000-fold, from about 7-fold to about 10,000-fold, from about 8-fold to about 10,000-fold, from about 9-fold to about 10,000-fold, from about 10-fold to about 10,000-fold, from about 11-fold to about 10,000-fold, from about 12-fold to about 10,000-fold, from about 14-fold to about 10,000-fold, from about 16-fold to about 10,000-fold, from about 20-fold to about 10,000-fold, from about 24-fold to about 10,000-fold, from about 30-fold to about 10,000-fold, from about 35-fold to about 10,000-fold, from about 40-fold to about 10,000-fold, from about 50-fold to about 10,000-fold, from about 60-fold to about 10,000-fold, from about 70-fold to about 10,000-fold, from about 80-fold to about 10,000-fold, from about 90-fold to about 10,000-fold, from about 100-fold to about 10,000-fold, from about 150-fold to about 10,000-fold, from about 200-fold to about 10,000-fold, from about 250-fold to about 10,000-fold, from about 300-fold to about 10,000-fold, from about 400-fold to about 10,000-fold, from about 500-fold to about 10,000-fold, from about 600-fold to about 10,000-fold, from about 700-fold to about 10,000-fold, from about 800-fold to about 10,000-fold, from about 900-fold to about 10,000-fold, or from about 1,000-fold to about 10,000-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 1,000-fold, from about 2-fold to about 1,000-fold, from about 3-fold to about 1,000-fold, from about 4-fold to about 1,000-fold, from about 5-fold to about 1,000-fold, from about 6-fold to about 1,000-fold, from about 7-fold to about 1,000-fold, from about 8-

fold to about 1,000-fold, from about 9-fold to about 1,000-fold, from about 10-fold to about 1,000-fold, from about 11-fold to about 1,000-fold, from about 12-fold to about 1,000-fold, from about 14-fold to about 1,000-fold, from about 16-fold to about 1,000-fold, from about 20-fold to about 1,000-fold, from about 24-fold to about 1,000-fold, from about 30-fold to about 1,000-fold, from about 35-fold to about 1,000-fold, from about 40-fold to about 1,000-fold, from about 50-fold to about 1,000-fold, from about 60-fold to about 1,000-fold, from about 70-fold to about 1,000-fold, from about 80-fold to about 1,000-fold, from about 90-fold to about 1,000-fold, from about 100-fold to about 1,000-fold, from about 150-fold to about 1,000-fold, from about 200-fold to about 1,000-fold, from about 250-fold to about 1,000-fold, from about 300-fold to about 1,000-fold, from about 400-fold to about 1,000-fold, from about 500-fold to about 1,000-fold, from about 600-fold to about 1,000-fold, from about 700-fold to about 1,000-fold, from about 800-fold to about 1,000-fold, or from about 900-fold to about 1,000-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 900-fold, from about 2-fold to about 900-fold, from about 3-fold to about 900-fold, from about 4-fold to about 900-fold, from about 5-fold to about 900-fold, from about 6-fold to about 900-fold, from about 7-fold to about 900-fold, from about 8-fold to about 900-fold, from about 9-fold to about 900-fold, from about 10-fold to about 900-fold, from about 11-fold to about 900-fold, from about 12-fold to about 900-fold, from about 14-fold to about 900-fold, from about 16-fold to about 900-fold, from about 20-fold to about 900-fold, from about 24-fold to about 900-fold, from about 30-fold to about 900-fold, from about 35-fold to about 900-fold, from about 40-fold to about 900-fold, from about 50-fold to about 900-fold, from about 60-fold to about 900-fold, from about 70-fold to about 900-fold, from about 80-fold to about 900-fold, from about 90-fold to about 900-fold, from about 100-fold to about 900-fold, from about 150-fold to about 900-fold, from about 200-fold to about 900-fold, from about 250-fold to about 900-fold, from about 300-fold to about 900-fold, from about 400-fold to about 900-fold, from about 500-fold to about 900-fold, from about 600-fold to about 900-fold, from about 700-fold to about 900-fold, or from about 800-fold to about 900-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 800-fold, from about 2-fold to about 800-fold, from about 3-fold to about 800-fold, from about 4-fold to about 800-fold, from about 5-fold to about 800-fold, from about 6-fold to about 800-fold, from about 7-fold to about 800-fold, from about 8-fold to about 800-fold,

from about 9-fold to about 800-fold, from about 10-fold to about 800-fold, from about 11-fold to about 800-fold, from about 12-fold to about 800-fold, from about 14-fold to about 800-fold, from about 16-fold to about 800-fold, from about 20-fold to about 800-fold, from about 24-fold to about 800-fold, from about 30-fold to about 800-fold, from about 35-fold

5       to about 800-fold, from about 40-fold to about 800-fold, from about 50-fold to about 800-fold, from about 60-fold to about 800-fold, from about 70-fold to about 800-fold, from about 80-fold to about 800-fold, from about 90-fold to about 800-fold, from about 100-fold to about 800-fold, from about 150-fold to about 800-fold, from about 200-fold to about 800-fold, from about 250-fold to about 800-fold, from about 300-fold to about 800-fold, from

10      about 400-fold to about 800-fold, from about 500-fold to about 800-fold, from about 600-fold to about 800-fold, or from about 700-fold to about 800-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 700-fold, from about 2-fold to about 700-fold, from about 3-fold to about 700-fold, from about 4-fold to about 700-fold, from about 5-fold to about 700-fold, from about 6-fold

15      to about 700-fold, from about 7-fold to about 700-fold, from about 8-fold to about 700-fold, from about 9-fold to about 700-fold, from about 10-fold to about 700-fold, from about 11-fold to about 700-fold, from about 12-fold to about 700-fold, from about 14-fold to about 700-fold, from about 16-fold to about 700-fold, from about 20-fold to about 700-fold, from about 24-fold to about 700-fold, from about 30-fold to about 700-fold, from about 35-fold

20      to about 700-fold, from about 40-fold to about 700-fold, from about 50-fold to about 700-fold, from about 60-fold to about 700-fold, from about 70-fold to about 700-fold, from about 80-fold to about 700-fold, from about 90-fold to about 700-fold, from about 100-fold to about 700-fold, from about 150-fold to about 700-fold, from about 200-fold to about 700-fold, from about 250-fold to about 700-fold, from about 300-fold to about 700-fold, from

25      about 400-fold to about 700-fold, from about 500-fold to about 700-fold, or from about 600-fold to about 700-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 600-fold, from about 2-fold to about 600-fold, from about 3-fold to about 600-fold, from about 4-fold to about 600-fold, from about 5-fold to about 600-fold, from about 6-fold

30      to about 600-fold, from about 7-fold to about 600-fold, from about 8-fold to about 600-fold, from about 9-fold to about 600-fold, from about 10-fold to about 600-fold, from about 11-fold to about 600-fold, from about 12-fold to about 600-fold, from about 14-fold to about 600-fold, from about 16-fold to about 600-fold, from about 20-fold to about 600-fold, from

about 24-fold to about 600-fold, from about 30-fold to about 600-fold, from about 35-fold to about 600-fold, from about 40-fold to about 600-fold, from about 50-fold to about 600-fold, from about 60-fold to about 600-fold, from about 70-fold to about 600-fold, from about 80-fold to about 600-fold, from about 90-fold to about 600-fold, from about 100-fold to about 600-fold, from about 150-fold to about 600-fold, from about 200-fold to about 600-fold, from about 250-fold to about 600-fold, from about 300-fold to about 600-fold, from about 400-fold to about 600-fold, or from about 500-fold to about 600-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 500-fold, from about 2-fold to about 500-fold, from about 3-fold to about 500-fold, from about 4-fold to about 500-fold, from about 5-fold to about 500-fold, from about 6-fold to about 500-fold, from about 7-fold to about 500-fold, from about 8-fold to about 500-fold, from about 9-fold to about 500-fold, from about 10-fold to about 500-fold, from about 11-fold to about 500-fold, from about 12-fold to about 500-fold, from about 14-fold to about 500-fold, from about 16-fold to about 500-fold, from about 20-fold to about 500-fold, from about 24-fold to about 500-fold, from about 30-fold to about 500-fold, from about 35-fold to about 500-fold, from about 40-fold to about 500-fold, from about 50-fold to about 500-fold, from about 60-fold to about 500-fold, from about 70-fold to about 500-fold, from about 80-fold to about 500-fold, from about 90-fold to about 500-fold, from about 100-fold to about 500-fold, from about 150-fold to about 500-fold, from about 200-fold to about 500-fold, from about 250-fold to about 500-fold, from about 300-fold to about 500-fold, or from about 400-fold to about 500-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 400-fold, from about 2-fold to about 400-fold, from about 3-fold to about 400-fold, from about 4-fold to about 400-fold, from about 5-fold to about 400-fold, from about 6-fold to about 400-fold, from about 7-fold to about 400-fold, from about 8-fold to about 400-fold, from about 9-fold to about 400-fold, from about 10-fold to about 400-fold, from about 11-fold to about 400-fold, from about 12-fold to about 400-fold, from about 14-fold to about 400-fold, from about 16-fold to about 400-fold, from about 20-fold to about 400-fold, from about 24-fold to about 400-fold, from about 30-fold to about 400-fold, from about 35-fold to about 400-fold, from about 40-fold to about 400-fold, from about 50-fold to about 400-fold, from about 60-fold to about 400-fold, from about 70-fold to about 400-fold, from about 80-fold to about 400-fold, from about 90-fold to about 400-fold, from about 100-fold

to about 400-fold, from about 150-fold to about 400-fold, from about 200-fold to about 400-fold, from about 250-fold to about 400-fold, or from about 300-fold to about 400-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 300-fold, from about 2-fold to about 300-fold, from about 3-fold to about 300-fold, from about 4-fold to about 300-fold, from about 5-fold to about 300-fold, from about 6-fold to about 300-fold, from about 7-fold to about 300-fold, from about 8-fold to about 300-fold, from about 9-fold to about 300-fold, from about 10-fold to about 300-fold, from about 11-fold to about 300-fold, from about 12-fold to about 300-fold, from about 14-fold to about 300-fold, from about 16-fold to about 300-fold, from about 20-fold to about 300-fold, from about 24-fold to about 300-fold, from about 30-fold to about 300-fold, from about 35-fold to about 300-fold, from about 40-fold to about 300-fold, from about 50-fold to about 300-fold, from about 60-fold to about 300-fold, from about 70-fold to about 300-fold, from about 80-fold to about 300-fold, from about 90-fold to about 300-fold, from about 100-fold to about 300-fold, from about 150-fold to about 300-fold, from about 200-fold to about 300-fold, or from about 250-fold to about 300-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 200-fold, from about 2-fold to about 200-fold, from about 3-fold to about 200-fold, from about 4-fold to about 200-fold, from about 5-fold to about 200-fold, from about 6-fold to about 200-fold, from about 7-fold to about 200-fold, from about 8-fold to about 200-fold, from about 9-fold to about 200-fold, from about 10-fold to about 200-fold, from about 11-fold to about 200-fold, from about 12-fold to about 200-fold, from about 14-fold to about 200-fold, from about 16-fold to about 200-fold, from about 20-fold to about 200-fold, from about 24-fold to about 200-fold, from about 30-fold to about 200-fold, from about 35-fold to about 200-fold, from about 40-fold to about 200-fold, from about 50-fold to about 200-fold, from about 60-fold to about 200-fold, from about 70-fold to about 200-fold, from about 80-fold to about 200-fold, from about 90-fold to about 200-fold, from about 100-fold to about 200-fold, or from about 150-fold to about 200-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 100-fold, from about 2-fold to about 100-fold, from about 3-fold to about 100-fold, from about 4-fold to about 100-fold, from about 5-fold to about 100-fold, from about 6-fold to about 100-fold, from about 7-fold to about 100-fold, from about 8-fold to about 100-fold, from about 9-fold to about 100-fold, from about 10-fold to about 100-fold, from about 11-fold to about 100-fold, from about 12-fold to about 100-fold, from about 14-fold to about

100-fold, from about 16-fold to about 100-fold, from about 20-fold to about 100-fold, from about 24-fold to about 100-fold, from about 30-fold to about 100-fold, from about 35-fold to about 100-fold, from about 40-fold to about 100-fold, from about 50-fold to about 100-fold, from about 60-fold to about 100-fold, from about 70-fold to about 100-fold, from about 80-fold to about 100-fold, or from about 90-fold to about 100-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 90-fold, from about 2-fold to about 90-fold, from about 3-fold to about 90-fold, from about 4-fold to about 90-fold, from about 5-fold to about 90-fold, from about 6-fold to about 90-fold, from about 7-fold to about 90-fold, from about 8-fold to about 90-fold, from about 9-fold to about 90-fold, from about 10-fold to about 90-fold, from about 11-fold to about 90-fold, from about 12-fold to about 90-fold, from about 14-fold to about 90-fold, from about 16-fold to about 90-fold, from about 20-fold to about 90-fold, from about 24-fold to about 90-fold, from about 30-fold to about 90-fold, from about 35-fold to about 90-fold, from about 40-fold to about 90-fold, from about 50-fold to about 90-fold, from about 60-fold to about 90-fold, from about 70-fold to about 90-fold, or from about 80-fold to about 90-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 80-fold, from about 2-fold to about 80-fold, from about 3-fold to about 80-fold, from about 4-fold to about 80-fold, from about 5-fold to about 80-fold, from about 6-fold to about 80-fold, from about 7-fold to about 80-fold, from about 8-fold to about 80-fold, from about 9-fold to about 80-fold, from about 10-fold to about 80-fold, from about 11-fold to about 80-fold, from about 12-fold to about 80-fold, from about 14-fold to about 80-fold, from about 16-fold to about 80-fold, from about 20-fold to about 80-fold, from about 24-fold to about 80-fold, from about 30-fold to about 80-fold, from about 35-fold to about 80-fold, from about 40-fold to about 80-fold, from about 50-fold to about 80-fold, from about 60-fold to about 80-fold, or from about 70-fold to about 80-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 70-fold, from about 2-fold to about 70-fold, from about 3-fold to about 70-fold, from about 4-fold to about 70-fold, from about 5-fold to about 70-fold, from about 6-fold to about 70-fold, from about 7-fold to about 70-fold, from about 8-fold to about 70-fold, from about 9-fold to about 70-fold, from about 10-fold to about 70-fold, from about 11-fold to about 70-fold, from about 12-fold to about 70-fold, from about 14-fold to about 70-fold, from about 16-fold to about 70-fold, from about 20-fold to about 70-fold, from about 24-

fold to about 70-fold, from about 30-fold to about 70-fold, from about 35-fold to about 70-fold, from about 40-fold to about 70-fold, from about 50-fold to about 70-fold, or from about 60-fold to about 70-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 60-fold, from about 2-fold to about 60-fold, from about 3-fold to about 60-fold, from about 4-fold to about 60-fold, from about 5-fold to about 60-fold, from about 6-fold to about 60-fold, from about 7-fold to about 60-fold, from about 8-fold to about 60-fold, from about 9-fold to about 60-fold, from about 10-fold to about 60-fold, from about 11-fold to about 60-fold, from about 12-fold to about 60-fold, from about 14-fold to about 60-fold, from about 16-fold to about 60-fold, from about 20-fold to about 60-fold, from about 24-fold to about 60-fold, from about 30-fold to about 60-fold, from about 35-fold to about 60-fold, from about 40-fold to about 60-fold, or from about 50-fold to about 60-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 50-fold, from about 2-fold to about 50-fold, from about 3-fold to about 50-fold, from about 4-fold to about 50-fold, from about 5-fold to about 50-fold, from about 6-fold to about 50-fold, from about 7-fold to about 50-fold, from about 8-fold to about 50-fold, from about 9-fold to about 50-fold, from about 10-fold to about 50-fold, from about 11-fold to about 50-fold, from about 12-fold to about 50-fold, from about 14-fold to about 50-fold, from about 16-fold to about 50-fold, from about 20-fold to about 50-fold, from about 24-fold to about 50-fold, from about 30-fold to about 50-fold, from about 35-fold to about 50-fold, or from about 40-fold to about 50-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 40-fold, from about 2-fold to about 40-fold, from about 3-fold to about 40-fold, from about 4-fold to about 40-fold, from about 5-fold to about 40-fold, from about 6-fold to about 40-fold, from about 7-fold to about 40-fold, from about 8-fold to about 40-fold, from about 9-fold to about 40-fold, from about 10-fold to about 40-fold, from about 11-fold to about 40-fold, from about 12-fold to about 40-fold, from about 14-fold to about 40-fold, from about 16-fold to about 40-fold, from about 20-fold to about 40-fold, from about 24-fold to about 40-fold, from about 30-fold to about 40-fold, or from about 35-fold to about 40-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 30-fold, from about 2-fold to about 30-fold, from about 3-fold to about 30-fold, from about 4-fold to about 30-fold, from about 5-fold to about 30-fold, from about 6-fold to

130

about 30-fold, from about 7-fold to about 30-fold, from about 8-fold to about 30-fold, from about 9-fold to about 30-fold, from about 10-fold to about 30-fold, from about 11-fold to about 30-fold, from about 12-fold to about 30-fold, from about 14-fold to about 30-fold, from about 16-fold to about 30-fold, from about 20-fold to about 30-fold, or from about 24-fold to about 30-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 20-fold, from about 2-fold to about 20-fold, from about 3-fold to about 20-fold, from about 4-fold to about 20-fold, from about 5-fold to about 20-fold, from about 6-fold to about 20-fold, from about 7-fold to about 20-fold, from about 8-fold to about 20-fold, from about 9-fold to about 20-fold, from about 10-fold to about 20-fold, from about 11-fold to about 20-fold, from about 12-fold to about 20-fold, from about 14-fold to about 20-fold, or from about 16-fold to about 20-fold. The disclosed method can produce, for example, amplification of from about 1-fold to about 10-fold, from about 2-fold to about 10-fold, from about 3-fold to about 10-fold, from about 4-fold to about 10-fold, from about 5-fold to about 10-fold, from about 6-fold to about 10-fold, from about 7-fold to about 10-fold, from about 8-fold to about 10-fold, or from about 9-fold to about 10-fold.

The disclosed method can produce, for example, amplification of from about 1-fold to about 9-fold, from about 2-fold to about 9-fold, from about 3-fold to about 9-fold, from about 4-fold to about 9-fold, from about 5-fold to about 9-fold, from about 6-fold to about 9-fold, from about 7-fold to about 9-fold, or from about 8-fold to about 9-fold. The disclosed method can produce, for example, amplification of from about 1-fold to about 8-fold, from about 2-fold to about 8-fold, from about 3-fold to about 8-fold, from about 4-fold to about 8-fold, from about 5-fold to about 8-fold, from about 6-fold to about 8-fold, or from about 7-fold to about 8-fold. The disclosed method can produce, for example, amplification of from about 1-fold to about 7-fold, from about 2-fold to about 7-fold, from about 3-fold to about 7-fold, from about 4-fold to about 7-fold, from about 5-fold to about 7-fold, or from about 6-fold to about 7-fold. The disclosed method can produce, for example, amplification of from about 1-fold to about 6-fold, from about 2-fold to about 6-fold, from about 3-fold to about 6-fold, from about 4-fold to about 6-fold, or from about 5-fold to about 6-fold. The disclosed method can produce, for example, amplification of from about 1-fold to about 5-fold, from about 2-fold to about 5-fold, from about 3-fold to about 5-fold, from about 4-fold to about 5-fold, from about 1-fold to about 4-fold, from about 2-

fold to about 4-fold, from about 3-fold to about 4-fold, from about 1-fold to about 3-fold, from about 2-fold to about 3-fold, or from about 1-fold to about 2-fold.

**B. Primer Selection**

Primers for use in the disclosed method can be selected for their ability to produce high quality amplification products. Such primers are particularly useful in the disclosed method. Where more than one primer is used in the disclosed method, all of the primers can be selected primers or some of the primers can be selected primers. Any useful criteria can be used for primer selection. Useful criteria include the quality of amplification products, such as the locus representation, the sequence representation and the amplification bias, and a lack of negative characteristics, such as a lack or minimization of production of amplification artifacts. Primers that meet given selection criteria (or a selection criterion) are referred to herein as selected primers (for those selection criteria). Primers that do not meet the given selection criteria (or selection criterion) are referred to herein as non-selected primers (for those selection criteria). Both selected and non-selected primers can be used together in the disclosed method, although use of selected primers is preferred.

Selected primers meeting different selection criteria can be used together in the disclosed method. That is, the primers used in a given amplification reaction need not all have the same capabilities or meet the same criteria. Similarly, non-selected primers failing to meet different selection criteria can be included or excluded from use in the disclosed method. That is, primers not used (or used) need not lack the same capabilities or fail to meet the same criteria. Selected primers meeting a selection criterion, selection criteria, or a combination of different selection criteria, can be used with non-selected primers failing to meet the same or a different selection criterion, selection criteria, or a combination of the same or different selection criteria.

The disclosed method thus can be performed with one or more selected primers. The disclosed method can also be performed with one or more selected primers and one or more non-selected primers. Whether a primer is a selected primer or a non-selected primer can be determined by testing the primer for the selection criterion or criteria. Thus, for example, the primer can be tested in a form of the disclosed method. Such a method could use a nucleic acid sample of interest, such as a nucleic acid sample having relevant characteristics. A nucleic acid sample used for this purpose is referred to herein as a selection nucleic acid sample. Particularly useful selection nucleic acid samples are nucleic acid samples of the same type that the selected primers will be used to amplify. Thus, a

132

human genomic nucleic acid sample can be used as the selection sample for selecting primers to be used to amplify human genomic DNA. Also useful as selection nucleic acid samples are nucleic acid samples that can be used as standards for selecting primers to be used to amplify a variety of different types of nucleic acid samples. For example, a yeast

5    genomic nucleic acid sample can be used as a selection nucleic acid sample for selecting primers in general. Such a selection nucleic acid sample can set a benchmark for selection criteria. The sequence complexity of the selection nucleic acid sample can be important as, or an important factor in establishing, a selection criterion or selection criteria. Thus, for example, a certain quality of amplification product form a nucleic acid sample of a given

10   sequence complexity can be required. The selection nucleic acid sample can have any level of sequence complexity. For example, the selection nucleic acid sample can have any of the sequence complexity levels described elsewhere herein. In general, the higher the sequence complexity of the selection nucleic acid sample, the lower the quality that can be required or allowed for the selection criteria.

15          For selection of primers, for example, the primer can be brought into contact with a DNA polymerase and a selection nucleic acid sample and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample. The results can then be compared to the selection criterion or criteria.

            A primer can be selected based on producing a certain level or range of replication

20   of nucleic acid sequences in a selection nucleic acid sample. Any replication level can be used. For example, any of the replication levels described elsewhere herein can be used as the selection criterion. A selected primer can produce, for example, replication of at least 0.01% of the nucleic acid sequences in the nucleic acid sample, at least 0.1% of the nucleic acid sequences in the nucleic acid sample, at least 1% of the nucleic acid sequences in the

25   nucleic acid sample, at least 5% of the nucleic acid sequences in the nucleic acid sample, at least 10% of the nucleic acid sequences in the nucleic acid sample, at least 20% of the nucleic acid sequences in the nucleic acid sample, at least 30% of the nucleic acid sequences in the nucleic acid sample, at least 40% of the nucleic acid sequences in the nucleic acid sample, at least 50% of the nucleic acid sequences in the nucleic acid sample,

30   at least 60% of the nucleic acid sequences in the nucleic acid sample, at least 70% of the nucleic acid sequences in the nucleic acid sample, at least 80% of the nucleic acid sequences in the nucleic acid sample, at least 90% of the nucleic acid sequences in the nucleic acid sample, at least 95% of the nucleic acid sequences in the nucleic acid sample,

at least 96% of the nucleic acid sequences in the nucleic acid sample, at least 97% of the nucleic acid sequences in the nucleic acid sample, at least 98% of the nucleic acid sequences in the nucleic acid sample, or at least 99% of the nucleic acid sequences in the nucleic acid sample.

5          A primer can be selected based on producing a certain level or range of amplification of nucleic acid sequences in a selection nucleic acid sample. Any amplification level can be used. For example, any of the amplification levels described elsewhere herein can be used as the selection criterion. A selected primer can produce, for example, amplification of about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-

10        fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 14-fold, about 16-fold, about 20-fold, about 24-fold, about 30-fold, about 35-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, about 100-fold, about 150-fold, about 200-fold, about 250-fold, about 300-fold, about 400-fold, about 500-fold, about 600-fold, about 700-fold, about 800-fold, about

15        900-fold, about 1,000-fold, about 10,000-fold, about 100,000-fold, about 1,000,000-fold, about 10,000,000-fold, or about 100,000,000-fold. Fold amplification refers to the number of copies generated of the template being amplified. For example, if 1 ug of DNA is generated from 1 ng of template, the level of amplification is 1,000-fold.

          A selected primer can produce, for example, amplification of at least 2-fold, at least

20        3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 11-fold, at least 12-fold, at least 14-fold, at least 16-fold, at least 20-fold, at least 24-fold, at least 30-fold, at least 35-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, at least 100-fold, at least 150-fold, at least 200-fold, at least 250-fold, at least 300-fold, at least 400-fold, at least

25        500-fold, at least 600-fold, at least 700-fold, at least 800-fold, at least 900-fold, at least 1,000-fold, at least 10,000-fold, at least 100,000-fold, at least 1,000,000-fold, at least 10,000,000-fold, or at least 100,000,000-fold.

          A selected primer can produce, for example, amplification bias of at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at

30        least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 11-fold, at least about 12-fold, at least about 14-fold, at least about 16-fold, at least about 20-fold, at least about 24-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 50-fold, at least about 60-fold, at least about 70-fold, at least

about 80-fold, at least about 90-fold, at least about 100-fold, at least about 150-fold, at least about 200-fold, at least about 250-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 600-fold, at least about 700-fold, at least about 800-fold, at least about 900-fold, at least about 1,000-fold, at least about 10,000-fold, at least about

5       100,000-fold, at least about 1,000,000-fold, at least about 10,000,000-fold, or at least about 100,000,000-fold.

A primer can be selected based on producing a certain level or range of amplification bias. Any amplification bias can be used. For example, any of the amplification biases described elsewhere herein can be used as the selection criterion. A

10      selected primer can produce, for example, an amplification bias of 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 14-fold, 16-fold, 20-fold, 24-fold, 30-fold, 35-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, 200-fold, 250-fold, or 300-fold. A selected primer can produce, for example, an amplification bias of about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold,

15      about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 14-fold, about 16-fold, about 20-fold, about 24-fold, about 30-fold, about 35-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, about 100-fold, about 150-fold, about 200-fold, about 250-fold, or about 300-fold. A selected primer can produce, for example, an amplification bias of less than 2-fold, less than

20      3-fold, less than 4-fold, less than 5-fold, less than 6-fold, less than 7-fold, less than 8-fold, less than 9-fold, less than 10-fold, less than 11-fold, less than 12-fold, less than 14-fold, less than 16-fold, less than 20-fold, less than 24-fold, less than 30-fold, less than 35-fold, less than 40-fold, less than 50-fold, less than 60-fold, less than 70-fold, less than 80-fold, less than 90-fold, less than 100-fold, less than 150-fold, less than 200-fold, less than 250-fold, or

25      less than 300-fold.

A selected primer can produce, for example, an amplification bias of less than about 2-fold, less than about 3-fold, less than about 4-fold, less than about 5-fold, less than about 6-fold, less than about 7-fold, less than about 8-fold, less than about 9-fold, less than about 10-fold, less than about 11-fold, less than about 12-fold, less than about 14-fold, less than

30      about 16-fold, less than about 20-fold, less than about 24-fold, less than about 30-fold, less than about 35-fold, less than about 40-fold, less than about 50-fold, less than about 60-fold, less than about 70-fold, less than about 80-fold, less than about 90-fold, less than about 100-fold, less than about 150-fold, less than about 200-fold, less than about 250-fold, or less

than about 300-fold. These amplification biases can be, for example, for any number of loci or target sequences, such as, for example, a number of loci and/or target sequences described elsewhere herein.

A primer can be selected based on producing a certain level or range of sequence representation. Any sequence representation can be used. For example, any of the sequence representations described elsewhere herein can be used as the selection criterion. A selected primer can produce, for example, a sequence representation of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100%. A selected primer can produce, for example, a sequence representation of less than 500%, less than 400%, less than 300%, less than 250%, less than 200%, less than 190%, less than 180%, less than 170%, less than 160%, less than 150%, less than 140%, less than 130%, less than 120%, or less than 110%. These sequence representations can be, for example, for any number of target sequences, such as, for example, a number of target sequences described elsewhere herein.

A primer can be selected based on producing a certain level or range of locus representation. Any locus representation can be used. For example, any of the locus representations described elsewhere herein can be used as the selection criterion. A selected primer can produce, for example, a locus representation of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100%. A selected primer can produce, for example, a locus representation of less than 500%, less than 400%, less than 300%, less than 250%, less than 200%, less than 190%, less than 180%, less than 170%, less than 160%, less than 150%, less than 140%, less than 130%, less than 120%, or less than 110%. These locus representations can be, for example, for any number of loci, such as, for example, a number of loci described elsewhere herein.

Primers can also be selected as groups of primers. That is, whether the group of primers, when used together, exhibit the selection criterion or criteria can be tested. This can be accomplished in all the ways described herein for selection of single primers. Thus, for example, the group of primers can be brought into contact with a DNA polymerase and a selection nucleic acid sample and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample.

Non-selected primers can be identified in the same way using similar criteria as selected primers are identified. The difference is that the non-selected primers fail to meet a

selection criterion or selection criteria. It is understood however, that such failure to meet a criterion or criteria can be expressed as having a certain characteristic or feature, just as in the case of selected primers. Such criteria can be referred to as non-selection criteria.

5          A non-selected primer can be identified based on producing or failing to produce a certain level or range of replication of nucleic acid sequences in a selection nucleic acid sample. Any replication level can be used as the standard. For example, any of the replication levels described elsewhere herein can be used as the non-selection criterion. A non-selected primer can produce, for example, replication of less than 0.01% of the nucleic acid sequences in the nucleic acid sample, less than 0.1% of the nucleic acid sequences in

10        the nucleic acid sample, less than 1% of the nucleic acid sequences in the nucleic acid sample, less than 5% of the nucleic acid sequences in the nucleic acid sample, less than 10% of the nucleic acid sequences in the nucleic acid sample, less than 20% of the nucleic acid sequences in the nucleic acid sample, less than 30% of the nucleic acid sequences in the nucleic acid sample, less than 40% of the nucleic acid sequences in the nucleic acid sample,

15        less than 50% of the nucleic acid sequences in the nucleic acid sample, less than 60% of the nucleic acid sequences in the nucleic acid sample, less than 70% of the nucleic acid sequences in the nucleic acid sample, less than 80% of the nucleic acid sequences in the nucleic acid sample, less than 90% of the nucleic acid sequences in the nucleic acid sample, less than 95% of the nucleic acid sequences in the nucleic acid sample, less than 96% of the

20        nucleic acid sequences in the nucleic acid sample, less than 97% of the nucleic acid sequences in the nucleic acid sample, less than 98% of the nucleic acid sequences in the nucleic acid sample, or less than 99% of the nucleic acid sequences in the nucleic acid sample.

          A non-selected primer can be identified based on producing or failing to produce a

25        certain level or range of amplification bias. Any amplification bias can be used as the standard. For example, any of the amplification biases described elsewhere herein can be used as the non-selection criterion. A non-selected primer can produce, for example, an amplification bias of 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 14-fold, 16-fold, 20-fold, 24-fold, 30-fold, 35-fold, 40-fold, 50-fold,

30        60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, 200-fold, 250-fold, or 300-fold. A non-selected primer can produce, for example, an amplification bias of about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 14-fold, about 16-fold,

about 20-fold, about 24-fold, about 30-fold, about 35-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, about 100-fold, about 150-fold, about 200-fold, about 250-fold, or about 300-fold. A non-selected primer can produce, for example, an amplification bias of more than 2-fold, more than 3-fold, more than 4-fold,

5      more than 5-fold, more than 6-fold, more than 7-fold, more than 8-fold, more than 9-fold, more than 10-fold, more than 11-fold, more than 12-fold, more than 14-fold, more than 16-fold, more than 20-fold, more than 24-fold, more than 30-fold, more than 35-fold, more than 40-fold, more than 50-fold, more than 60-fold, more than 70-fold, more than 80-fold, more than 90-fold, more than 100-fold, more than 150-fold, more than 200-fold, more than 250-

10     fold, or more than 300-fold.

A selected primer can produce, for example, an amplification bias of more than about 2-fold, more than about 3-fold, more than about 4-fold, more than about 5-fold, more than about 6-fold, more than about 7-fold, more than about 8-fold, more than about 9-fold, more than about 10-fold, more than about 11-fold, more than about 12-fold, more than

15     about 14-fold, more than about 16-fold, more than about 20-fold, more than about 24-fold, more than about 30-fold, more than about 35-fold, more than about 40-fold, more than about 50-fold, more than about 60-fold, more than about 70-fold, more than about 80-fold, more than about 90-fold, more than about 100-fold, more than about 150-fold, more than about 200-fold, more than about 250-fold, or more than about 300-fold. These

20     amplification biases can be, for example, for any number of loci or target sequences, such as, for example, a number of loci and/or target sequences described elsewhere herein.

A non-selected primer can be identified based on producing a certain level or range of sequence representation. Any sequence representation can be used as the standard. For example, any of the sequence representations described elsewhere herein can be used as the

25     non-selection criterion. A non-selected primer can produce, for example, a sequence representation of less than 0.1%, less than 1%, less than 10%, less than 20%, less than 30%, less than 40%, less than 50%, less than 60%, less than 70%, less than 80%, less than 90%, or less than 100%. A selected primer can produce, for example, a sequence representation of more than 500%, more than 400%, more than 300%, more than 250%, more than 200%,

30     more than 190%, more than 180%, more than 170%, more than 160%, more than 150%, more than 140%, more than 130%, more than 120%, or more than 110%. These sequence representations can be, for example, for any number of target sequences, such as, for example, a number of target sequences described elsewhere herein.

138

A non-selected primer can be identified based on producing a certain level or range of locus representation. Any locus representation can be used as the standard. For example, any of the locus representations described elsewhere herein can be used as the non-selection criterion. A non-selected primer can produce, for example, a locus representation of less

5      than 0.1%, less than 1%, less than 10%, less than 20%, less than 30%, less than 40%, less than 50%, less than 60%, less than 70%, less than 80%, less than 90%, or less than 100%. A selected primer can produce, for example, a locus representation of more than 500%, more than 400%, more than 300%, more than 250%, more than 200%, more than 190%, more than 180%, more than 170%, more than 160%, more than 150%, more than 140%,

10     more than 130%, more than 120%, or more than 110%. These locus representations can be, for example, for any number of loci, such as, for example, a number of loci described elsewhere herein.

For establishing potential for artifact production by a primer or group of primers, the primer or group of primers can be tested in a modified form of the disclosed method where

15     no nucleic acid sample is used. If, when, and at what level amplification products are observed in such an assay is a measure of the potential of the primer or group of primers to produce amplification artifacts. One criterion for low potential for artifact production is a long delay before amplification products are first observed in a reaction performed in the absence of a nucleic acid sample (or other template nucleic acids). Delays can be, for

20     example, to 125%, 150%, 175%, 200%, 225%, 250%, 275%, 300%, 325%, 350%, 375%, 400%, 425%, 450%, 475%, or 500% of the time where amplification products are first observed in a reaction having a nucleic acid sample. Delays can be, for example, to greater than 125%, greater than 150%, greater than 175%, greater than 200%, greater than 225%, greater than 250%, greater than 275%, greater than 300%, greater than 325%, greater than

25     350%, greater than 375%, greater than 400%, greater than 425%, greater than 450%, greater than 475%, or greater than 500% of the time where amplification products are first observed in a reaction having a nucleic acid sample.

## C. Whole Genome Strand Displacement Amplification

In one form of the method, referred to as whole genome strand displacement

30     amplification (WGSDA), a random or partially random set of primers is used to randomly prime a sample of genomic nucleic acid (or another sample of nucleic acid of high complexity). By choosing a sufficiently large set of primers of random or mostly random sequence, the primers in the set will be collectively, and randomly, complementary to

nucleic acid sequences distributed throughout nucleic acid in the sample. Amplification proceeds by replication with a processive polymerase initiated at each primer and continuing until spontaneous termination. A key feature of this method is the displacement of intervening primers during replication by the polymerase. In this way, multiple

5      overlapping copies of the entire genome can be synthesized in a short time.

Whole genome strand displacement amplification can be performed by (a) mixing a set of random or partially random primers with a genomic sample (or other nucleic acid sample of high complexity), to produce a primer-target sample mixture, and incubating the primer-target sample mixture under conditions that promote hybridization between the

10     primers and the genomic DNA in the primer-target sample mixture, and (b) mixing DNA polymerase with the primer-target sample mixture, to produce a polymerase-target sample mixture, and incubating the polymerase-target sample mixture under conditions that promote replication of the genomic DNA. Strand displacement replication is preferably accomplished by using a strand displacing DNA polymerase or a DNA polymerase in

15     combination with a compatible strand displacement factor.

The method has advantages over the polymerase chain reaction since it can be carried out under isothermal conditions. Other advantages of whole genome strand displacement amplification include a higher level of amplification than whole genome PCR, amplification is less sequence-dependent than PCR, a lack of re-annealing artifacts or gene

20     shuffling artifacts as can occur with PCR (since there are no cycles of denaturation and re-annealing), and a lower amplification bias than PCR-based genome amplification (bias of 3-fold for WGSDA versus 20- to 60-fold for PCR-based genome amplification).

Following amplification, the amplified sequences can be used for any purpose, such as uses known and established for PCR amplified sequences. For example, amplified

25     sequences can be detected using any of the conventional detection systems for nucleic acids such as detection of fluorescent labels, enzyme-linked detection systems, antibody-mediated label detection, and detection of radioactive labels. A key feature of the disclosed method is that amplification takes place not in cycles, but in a continuous, isothermal replication. This makes amplification less complicated and much more consistent in output. Strand

30     displacement allows rapid generation of multiple copies of a nucleic acid sequence or sample in a single, continuous, isothermal reaction.

It is preferred that the set of primers used for WGSDA be used at concentrations that allow the primers to hybridize at desired intervals within the nucleic acid sample. For

example, by using a set of primers at a concentration that allows them to hybridize, on average, every 4000 to 8000 bases, DNA replication initiated at these sites will extend to and displace strands being replicated from adjacent sites. It should be noted that the primers are not expected to hybridize to the target sequence at regular intervals. Rather, the average

5      interval will be a general function of primer concentration. Primers for WGSDA can also be formed from RNA present in the sample. By degrading endogenous RNA with RNase to generate a pool of random oligomers, the random oligomers can then be used by the polymerase for amplification of the DNA. This eliminates any need to add primers to the reaction. Alternatively, DNase digestion of biological samples can generate a pool of DNA

10     oligo primers for RNA dependent DNA amplification.

As in multiple strand displacement amplification, displacement of an adjacent strand makes it available for hybridization to another primer and subsequent initiation of another round of replication. The interval at which primers in the set of primers hybridize to the target sequence determines the level of amplification. For example, if the average interval

15     is short, adjacent strands will be displaced quickly and frequently. If the average interval is long, adjacent strands will be displaced only after long runs of replication.

It is preferred that the number of primers used for WGSDA allow the primers to hybridize at desired intervals within the nucleic acid sample. For example, by using a number of primers that allows them to hybridize, on average, every 4000 to 8000 bases,

20     DNA replication initiated at these sites will extend to and displace strands being replicated from adjacent sites. It should be noted that the primers are not expected to hybridize to nucleic acid molecules in the nucleic acid sample at regular intervals. Rather, the average interval will be a general function of the number of primers (as described elsewhere herein).

Displacement of an adjacent strand makes it available for hybridization to another

25     primer and subsequent initiation of another round of replication. The interval at which the primers hybridize to the nucleic acid molecules generally determines the level of amplification. For example, if the average interval is short, adjacent strands will be displaced quickly and frequently. If the average interval is long, adjacent strands will be displaced only after long runs of replication.

30     In the disclosed method, the DNA polymerase catalyzes primer extension and strand displacement in a processive strand displacement polymerization reaction that proceeds as long as desired. Preferred strand displacing DNA polymerases are bacteriophage $\phi$29 DNA polymerase (U.S. Patent Nos. 5,198,543 and 5,001,050 to Blanco *et al.*), large fragment Bst

DNA polymerase (Exo(-) Bst), exo(-)Bca DNA polymerase, and Sequenase. During strand displacement replication one may additionally include radioactive, or modified nucleotides such as bromodeoxyuridine triphosphate, in order to label the DNA generated in the reaction. Alternatively, one may include suitable precursors that provide a binding moiety

5      such as biotinylated nucleotides (Langer *et al.*, *Proc. Natl. Acad. Sci. USA* **78**:6633 (1981)).

Genome amplification using PCR, and uses for the amplified DNA, is described in Zhang *et al.*, *Proc. Natl. Acad. Sci. USA* **89**:5847-5851 (1992), Telenius *et al.*, *Genomics* **13**:718-725 (1992), Cheung *et al.*, *Proc. Natl. Acad. Sci. USA* **93**:14676-14679 (1996), and Kukasjaarvi *et al.*, *Genes, Chromosomes and Cancer* **18**:94-101 (1997). The uses of the

10     amplified DNA described in these publications are also generally applicable to DNA amplified using the disclosed methods. Whole Genome Strand Displacement Amplification, unlike PCR-based whole genome amplification, is suitable for haplotype analysis since WGSDA yields longer fragments than PCR-based whole genome amplification. PCR-based whole genome amplification is also less suitable for haplotype

15     analysis since each cycle in PCR creates an opportunity for priming events that result.in the association of distant sequences (in the genome) to be put together in the same fragment.

Long nucleic acid segments can be amplified in the disclosed method since there is no cycling which could interrupt continuous synthesis or allow the formation of artifacts due to rehybridization of replicated strands.

20     **D.  Multiple Strand Displacement Amplification**

In one preferred form of the method, referred to as multiple strand displacement amplification (MSDA), two sets of primers are used, a right set and a left set. Primers in the right set of primers each have a portion complementary to nucleotide sequences flanking one side of a target nucleotide sequence and primers in the left set of primers each have a

25     portion complementary to nucleotide sequences flanking the other side of the target nucleotide sequence. The primers in the right set are complementary to one strand of the nucleic acid molecule containing the target nucleotide sequence and the primers in the left set are complementary to the opposite strand. The 5' end of primers in both sets are distal to the nucleic acid sequence of interest when the primers are hybridized to the flanking

30     sequences in the nucleic acid molecule. Preferably, each member of each set has a portion complementary to a separate and non-overlapping nucleotide sequence flanking the target nucleotide sequence. Amplification proceeds by replication initiated at each primer and continuing through the target nucleic acid sequence. A key feature of this method is the

displacement of intervening primers during replication. Once the nucleic acid strands elongated from the right set of primers reaches the region of the nucleic acid molecule to which the left set of primers hybridizes, and vice versa, another round of priming and replication will take place. This allows multiple copies of a nested set of the target nucleic acid sequence to be synthesized in a short period of time.

Multiple strand displacement amplification can be performed by (a) mixing a set of primers with a target sample, to produce a primer-target sample mixture, and incubating the primer-target sample mixture under conditions that promote hybridization between the primers and the target sequence in the primer-target sample mixture, and (b) mixing DNA polymerase with the primer-target sample mixture, to produce a polymerase-target sample mixture, and incubating the polymerase-target sample mixture under conditions that promote replication of the target sequence. Strand displacement replication is preferably accomplished by using a strand displacing DNA polymerase or a DNA polymerase in combination with a compatible strand displacement factor.

By using a sufficient number of primers in the right and left sets, only a few rounds of replication are required to produce hundreds of thousands of copies of the nucleic acid sequence of interest. For example, it can be estimated that, using right and left primer sets of 26 primers each, 200,000 copies of a 5000 nucleotide amplification target can be produced in 10 minutes (representing just four rounds of priming and replication). It can also be estimated that, using right and left primer sets of 26 primers each, 200,000 copies of a 47,000 nucleotide amplification target can be produced in 60 minutes (again representing four rounds of priming and replication). These calculations are based on a polymerase extension rate of 50 nucleotides per second. It is emphasized that reactions are continuous and isothermal -- no cycling is required.

The disclosed method has advantages over the polymerase chain reaction since it can be carried out under isothermal conditions. No thermal cycling is needed because the polymerase at the head of an elongating strand (or a compatible strand-displacement factor) will displace, and thereby make available for hybridization, the strand ahead of it. Other advantages of multiple strand displacement amplification include the ability to amplify very long nucleic acid segments (on the order of 50 kilobases) and rapid amplification of shorter segments (10 kilobases or less). Long nucleic acid segments can be amplified in the disclosed method since there is no cycling which could interrupt continuous synthesis or allow the formation of artifacts due to rehybridization of replicated strands. In multiple

strand displacement amplification, single priming events at unintended sites will not lead to artifactual amplification at these sites (since amplification at the intended site will quickly outstrip the single strand replication at the unintended site).

In another form of the method, referred to as gene specific strand displacement amplification (GS-MSDA), target DNA is first digested with a restriction endonuclease. The digested fragments are then ligated end-to-end to form DNA circles. These circles can be monomers or concatemers. Two sets of primers are used for amplification, a right set and a left set. Primers in the right set of primers each have a portion complementary to nucleotide sequences flanking one side of a target nucleotide sequence and primers in the left set of primers each have a portion complementary to nucleotide sequences flanking the other side of the target nucleotide sequence. The primers in the right set are complementary to one strand of the nucleic acid molecule containing the target nucleotide sequence and the primers in the left set are complementary to the opposite strand. The primers are designed to cover all or part of the sequence needed to be amplified. Preferably, each member of each set has a portion complementary to a separate and non-overlapping nucleotide sequence flanking the target nucleotide sequence. Amplification proceeds by replication initiated at each primer and continuing through the target nucleic acid sequence. In one form of GS-MSDA, referred to as linear GS-MSDA, amplification is performed with a set of primers complementary to only one strand, thus amplifying only one of the strands. In another form of GS-MSDA, cDNA sequences can be circularized to form single stranded DNA circles. Amplification is then performed with a set of primers complementary to the single-stranded circular cDNA.

### E.  Multiple Strand Displacement Amplification of Concatenated DNA

In another form of the method, referred to as multiple strand displacement amplification of concatenated DNA (MSDA-CD), concatenated DNA is amplified. A preferred form of concatenated DNA is concatenated cDNA. Concatenated DNA can be amplified using a random or partially random set of primers, as in WGSDA, or using specific primers complementary to specific hybridization targets in the concatenated DNA. MSDA-CD is preferred for amplification of a complex mixture or sample of relatively short nucleic acid samples (that is, fragments generally in the range of 100 to 6,000 nucleotides). Messenger RNA is the most important example of such a complex mixture. MSDA-CD provides a means for amplifying all cDNAs in a cell is equal fashion. Because the concatenated cDNA can be amplified up to 5,000-fold, MSDA-CD will permit RNA

profiling analysis based on just a few cells. To perform MSDA-CD, DNA must first be subjected to a concatenation step. If an RNA sample (such as mRNA) is to be amplified, the RNA is first converted to a double-stranded cDNA using standard methods. The cDNA, or any other set of DNA fragments to be amplified, is then converted into a DNA
5   concatenate, preferably with incorporation of linkers.

**F. Multiple Strand Displacement Amplification of Damaged DNA**

Other forms of the disclosed method can involve amplification and repair of damaged DNA. Amplification of damaged DNA can be both difficult and provide unreliable results. For example, amplification of degraded or fragmented DNA will
10   produce truncated products and can result in allele dropout. Preparation of genomic DNA samples in particular can result in damage to the genomic DNA (for example, degradation and fragmentation). Damaged DNA and damaged DNA samples can be amplified and repaired in the disclosed method of amplifying damage DNA. The method generally works by hybridizing the ends of some DNA molecules in a damaged DNA sample to
15   complementary sequences in the sample. Because the DNA molecules providing the newly associated ends will have damage at different locations, priming from the annealed ends can result in replication of more complete fragments and can mediate repair of the damaged DNA (in the form of less damaged or undamaged replicated strands). Replication of the undamaged replicated strands by continued multiple displacement amplification produces
20   less damaged or undamaged amplified nucleic acids.

The method generally involves substantially denaturing a damaged DNA sample (generally via exposure to heat and alkaline conditions), removal or reduction of the denaturing conditions (such as by reduction of the pH and temperature of the denatured DNA sample), and replicating the DNA. The damaged DNA is repaired during replication
25   and the average length of DNA fragments is increased. For example, the average length of DNA fragments can be increase from, for example, 2 kb in the damaged DNA sample to, for example, 10 kb or greater for the replicated DNA. The amplified and repaired DNA is in better condition for analysis and testing than the damaged DNA sample. For example, this technique can provide consistent improvements in allele representation from damaged
30   DNA samples. This repair method can result in an overall improvement in amplification of damaged DNA by increasing the average length of the product, increasing the quality of the amplification products by 3-fold (by, for example, increasing the marker representation in the sample), and improving the genotyping of amplified products by lowering the frequency

of allelic dropout; all compared to the results when amplifying damaged DNA by other methods. The replication can be multiple displacement amplification. Denaturation of the DNA sample generally is carried out such that the DNA is not further damaged. This method can generally be combined or used with any of the disclosed amplification methods.

5        In some embodiments, the method of amplifying damaged DNA can involve exposing a damaged DNA sample to conditions that promote substantial denaturation of damaged DNA in the damaged DNA sample, thereby forming a denatured damaged DNA sample; altering the conditions to conditions that do not promote substantial denaturation of damaged DNA in the damaged DNA sample to form a stabilized denatured damaged DNA

10       sample; and incubating the annealed damaged DNA under conditions that promote replication of the damaged DNA. The annealed ends of the damaged DNA prime replication and replication of the damaged DNA results in repair of the replicated strands. The conditions that promote substantial denaturation of damaged DNA in the damaged DNA sample can be, for example, raising the pH of the damaged DNA sample and heating

15       the damaged DNA sample. The altering conditions can be, for example, reducing the pH of the denatured damaged DNA sample and cooling the damaged DNA sample. Raising the pH can be accomplished by exposing the damaged DNA sample to alkaline conditions. The altering conditions generally can be conditions that promote annealing of the ends of the transiently denatured damaged DNA to the substantially denatured damaged DNA. The

20       damaged DNA sample, the denatured damaged DNA sample, or both can also be exposed to ionic conditions by, for example, mixing the damaged DNA sample or denatured damaged DNA sample with an ionic solution or including salt(s) or other ions in the denaturing solution, the stabilization solution, or both.

In the method, the damaged DNA sample can be exposed to conditions that promote

25       substantial denaturation by, for example, mixing the damaged DNA sample with a denaturing solution and by heating the damaged DNA sample to a temperature and for a length of time that substantially denatures the damaged DNA in the damaged DNA sample. The temperature can be, for example, about 25°C to about 50°C and the length of time can be, for example, about 5 minutes or more. The pH of the denatured damaged DNA sample

30       can be reduced, for example, by mixing the denatured damaged DNA sample with a stabilization solution. The damaged DNA samples can be, for example, degraded DNA fragments of genomic DNA. Replication and repair of the damaged DNA can be

accomplished by incubating the damaged DNA in the presence of a DNA polymerase, such as φ29 DNA polymerase.

The damaged DNA sample can generally be slowly cooled in order to achieve the required annealing. For example, the damaged DNA sample can be cooled at a rate of, for example, about 0.1°C per minute or less, about 0.2°C per minute or less, about 0.3°C per minute or less, about 0.4°C per minute or less, about 0.5°C per minute or less, about 0.6°C per minute or less, about 0.7°C per minute or less, about 0.8°C per minute or less, about 0.9°C per minute or less, about 1°C per minute or less, about 1.0°C per minute or less, about 1.1°C per minute or less, about 1.2°C per minute or less, about 1.3°C per minute or less, about 1.4°C per minute or less, about 1.5°C per minute or less, about 1.6°C per minute or less, about 1.8°C per minute or less, about 2°C per minute or less, about 2.0°C per minute or less, about 2.2°C per minute or less, about 2.4°C per minute or less, about 2.6°C per minute or less, about 2.8°C per minute or less, about 3°C per minute or less, about 3.0°C per minute or less, about 3.5°C per minute or less, about 4°C per minute or less, about 4.0°C per minute or less, about 5.0°C per minute or less, about 0.1°C per minute, about 0.2°C per minute, about 0.3°C per minute, about 0.4°C per minute, about 0.5°C per minute, about 0.6°C per minute, about 0.7°C per minute, about 0.8°C per minute, about 0.9°C per minute, about 1°C per minute, about 1.0°C per minute, about 1.1°C per minute, about 1.2°C per minute, about 1.3°C per minute, about 1.4°C per minute, about 1.5°C per minute, about 1.6°C per minute, about 1.8°C per minute, about 2°C per minute, about 2.0°C per minute, about 2.2°C per minute, about 2.4°C per minute, about 2.6°C per minute, about 2.8°C per minute, about 3°C per minute, about 3.0°C per minute, about 3.5°C per minute, about 4°C per minute, about 4.0°C per minute, about 5.0°C per minute, 0.1°C per minute, 0.2°C per minute, 0.3°C per minute, 0.4°C per minute, 0.5°C per minute, 0.6°C per minute, 0.7°C per minute, 0.8°C per minute, 0.9°C per minute, 1°C per minute, 1.0°C per minute, 1.1°C per minute, 1.2°C per minute, 1.3°C per minute, 1.4°C per minute, 1.5°C per minute, 1.6°C per minute, 1.8°C per minute, 2°C per minute, 2.0°C per minute, 2.2°C per minute, 2.4°C per minute, 2.6°C per minute, 2.8°C per minute, 3°C per minute, 3.0°C per minute, 3.5°C per minute, 4°C per minute, 4.0°C per minute, or 5.0°C per minute.

The rate of cooling of the damaged DNA sample can also described in terms of the percent drop in temperature. Thus, cooling a damaged DNA sample that starts at 70°C at a rate of 1% per minute or less would be cooled by 0.7°C (or less) in the first minute and 1% (or less) of the resulting temperature in the next minute. The damaged DNA sample can be

cooled at a rate of, for example, about 0.1% per minute or less, about 0.2% per minute or less, about 0.3% per minute or less, about 0.4% per minute or less, about 0.5% per minute or less, about 0.6% per minute or less, about 0.7% per minute or less, about 0.8% per minute or less, about 0.9% per minute or less, about 1% per minute or less, about 1.0% per minute or less, about 1.1% per minute or less, about 1.2% per minute or less, about 1.3% per minute or less, about 1.4% per minute or less, about 1.5% per minute or less, about 1.6% per minute or less, about 1.8% per minute or less, about 2% per minute or less, about 2.0% per minute or less, about 2.2% per minute or less, about 2.4% per minute or less, about 2.6% per minute or less, about 2.8% per minute or less, about 3% per minute or less, about 3.0% per minute or less, about 3.5% per minute or less, about 4% per minute or less, about 4.0% per minute or less, about 5.0% per minute or less, about 0.1% per minute, about 0.2% per minute, about 0.3% per minute, about 0.4% per minute, about 0.5% per minute, about 0.6% per minute, about 0.7% per minute, about 0.8% per minute, about 0.9% per minute, about 1% per minute, about 1.0% per minute, about 1.1% per minute, about 1.2% per minute, about 1.3% per minute, about 1.4% per minute, about 1.5% per minute, about 1.6% per minute, about 1.8% per minute, about 2% per minute, about 2.0% per minute, about 2.2% per minute, about 2.4% per minute, about 2.6% per minute, about 2.8% per minute, about 3% per minute, about 3.0% per minute, about 3.5% per minute, about 4% per minute, about 4.0% per minute, about 5.0% per minute, 0.1% per minute, 0.2% per minute, 0.3% per minute, 0.4% per minute, 0.5% per minute, 0.6% per minute, 0.7% per minute, 0.8% per minute, 0.9% per minute, 1% per minute, 1.0% per minute, 1.1% per minute, 1.2% per minute, 1.3% per minute, 1.4% per minute, 1.5% per minute, 1.6% per minute, 1.8% per minute, 2% per minute, 2.0% per minute, 2.2% per minute, 2.4% per minute, 2.6% per minute, 2.8% per minute, 3% per minute, 3.0% per minute, 3.5% per minute, 4% per minute, 4.0% per minute, or 5.0% per minute.

The damaged DNA sample, the denatured damaged DNA sample, or both can also be exposed to ionic conditions by, for example, mixing the damaged DNA sample or denatured damaged DNA sample with an ionic solution or including salt(s) or other ions in the denaturing solution, the stabilization solution, or both. As used herein, ionic conditions refers to a state of increased ionic strength. Thus, exposure to ionic conditions refers to exposure to a higher ionic strength than existed in the sample or solution before exposure. This will be the result when, for example, a buffer or salt is added. A solution used to make such an addition can be referred to as an ionic solution. The ionic solution can be a salt

solution and can comprise one or more salts or other ions. Any suitable salt or ion can be used. The salt can be, for example, Tris-HCl, Tris-EDTA, soduim chloride, potassium chloride, magnesium chloride, sodium acetate, potassium acetate, magnesium acetate, or a combination. The Tris-HCl can be, for example, from pH 7.0 to 8.0. The salt can be Tris-EDTA. The ionic solution can be diluted, for example, 2 to 5 fold when mixed with the damaged DNA sample. The ionic solution can be mixed with the denatured damaged DNA sample prior to or during altering of the conditions.

Ionic conditions and the composition of ionic solutions generally can be can be specified by specifying a concentration of a buffer, salt, or other ion-forming compound. A combination of compounds can be used in an ionic solution or to create ionic conditions. The salt solution can comprise, for example, about 50 mM to about 500 mM Tris and about 1 mM to about 5 mM EDTA. Ionic solutions can have a salt, buffer or ion concentration of from about 1 mM to about 2 M, from about 1 mM to about 1 M, from about 1 mM to about 900 mM, from about 1 mM to about 800 mM, from about 1 mM to about 700 mM, from about 1 mM to about 600 mM, from about 1 mM to about 500 mM, from about 1 mM to about 400 mM, from about 1 mM to about 300 mM, from about 1 mM to about 250 mM, from about 1 mM to about 200 mM, from about 1 mM to about 150 mM, from about 1 mM to about 100 mM, from about 1 mM to about 80 mM, from about 1 mM to about 60 mM, from about 1 mM to about 50 mM, from about 1 mM to about 40 mM, from about 1 mM to about 30 mM, from about 1 mM to about 20 mM, from about 1 mM to about 10 mM, from about 1 mM to about 5 mM, from about 1 mM to about 2 mM, from about 2 mM to about 2 M, from about 2 mM to about 1 M, from about 2 mM to about 900 mM, from about 2 mM to about 800 mM, from about 2 mM to about 700 mM, from about 2 mM to about 600 mM, from about 2 mM to about 500 mM, from about 2 mM to about 400 mM, from about 2 mM to about 300 mM, from about 2 mM to about 250 mM, from about 2 mM to about 200 mM, from about 2 mM to about 150 mM, from about 2 mM to about 100 mM, from about 2 mM to about 80 mM, from about 2 mM to about 60 mM, from about 2 mM to about 50 mM, from about 2 mM to about 40 mM, from about 2 mM to about 30 mM, from about 2 mM to about 20 mM, from about 2 mM to about 10 mM, from about 2 mM to about 5 mM, from about 5 mM to about 2 M, from about 5 mM to about 1 M, from about 5 mM to about 900 mM, from about 5 mM to about 800 mM, from about 5 mM to about 700 mM, from about 5 mM to about 600 mM, from about 5 mM to about 500 mM, from about 5 mM to about 400 mM, from about 5 mM to about 300 mM, from about 5 mM to about 250 mM, from about 5

mM to about 200 mM, from about 5 mM to about 150 mM, from about 5 mM to about 100 mM, from about 5 mM to about 80 mM, from about 5 mM to about 60 mM, from about 5 mM to about 50 mM, from about 5 mM to about 40 mM, from about 5 mM to about 30 mM, from about 5 mM to about 20 mM, from about 5 mM to about 10 mM, from about 10 mM to about 2 M, from about 10 mM to about 1 M, from about 10 mM to about 900 mM, from about 10 mM to about 800 mM, from about 10 mM to about 700 mM, from about 10 mM to about 600 mM, from about 10 mM to about 500 mM, from about 10 mM to about 400 mM, from about 10 mM to about 300 mM, from about 10 mM to about 250 mM, from about 10 mM to about 200 mM, from about 10 mM to about 150 mM, from about 10 mM to about 100 mM, from about 10 mM to about 80 mM, from about 10 mM to about 60 mM, from about 10 mM to about 50 mM, from about 10 mM to about 40 mM, from about 10 mM to about 30 mM, from about 10 mM to about 20 mM, from about 20 mM to about 2 M, from about 20 mM to about 1 M, from about 20 mM to about 900 mM, from about 20 mM to about 800 mM, from about 20 mM to about 700 mM, from about 20 mM to about 600 mM, from about 20 mM to about 500 mM, from about 20 mM to about 400 mM, from about 20 mM to about 300 mM, from about 20 mM to about 250 mM, from about 20 mM to about 200 mM, from about 20 mM to about 150 mM, from about 20 mM to about 100 mM, from about 20 mM to about 80 mM, from about 20 mM to about 60 mM, from about 20 mM to about 50 mM, from about 20 mM to about 40 mM, from about 20 mM to about 30 mM, from about 30 mM to about 2 M, from about 30 mM to about 1 M, from about 30 mM to about 900 mM, from about 30 mM to about 800 mM, from about 30 mM to about 700 mM, from about 30 mM to about 600 mM, from about 30 mM to about 500 mM, from about 30 mM to about 400 mM, from about 30 mM to about 300 mM, from about 30 mM to about 250 mM, from about 30 mM to about 200 mM, from about 30 mM to about 150 mM, from about 30 mM to about 100 mM, from about 30 mM to about 80 mM, from about 30 mM to about 60 mM, from about 30 mM to about 50 mM, from about 30 mM to about 40 mM, from about 40 mM to about 2 M, from about 40 mM to about 1 M, from about 40 mM to about 900 mM, from about 40 mM to about 800 mM, from about 40 mM to about 700 mM, from about 40 mM to about 600 mM, from about 40 mM to about 500 mM, from about 40 mM to about 400 mM, from about 40 mM to about 300 mM, from about 40 mM to about 250 mM, from about 40 mM to about 200 mM, from about 40 mM to about 150 mM, from about 40 mM to about 100 mM, from about 40 mM to about 80 mM, from about 40 mM to about 60 mM, from about 40 mM to about 50 mM, from about 50 mM to about 2 M,

150

from about 50 mM to about 1 M, from about 50 mM to about 900 mM, from about 50 mM to about 800 mM, from about 50 mM to about 700 mM, from about 50 mM to about 600 mM, from about 50 mM to about 500 mM, from about 50 mM to about 400 mM, from about 50 mM to about 300 mM, from about 50 mM to about 250 mM, from about 50 mM to

5          about 200 mM, from about 50 mM to about 150 mM, from about 50 mM to about 100 mM, from about 50 mM to about 80 mM, from about 50 mM to about 60 mM, from about 60 mM to about 2 M, from about 60 mM to about 1 M, from about 60 mM to about 900 mM, from about 60 mM to about 800 mM, from about 60 mM to about 700 mM, from about 60 mM to about 600 mM, from about 60 mM to about 500 mM, from about 60 mM to about

10        400 mM, from about 60 mM to about 300 mM, from about 60 mM to about 250 mM, from about 60 mM to about 200 mM, from about 60 mM to about 150 mM, from about 60 mM to about 100 mM, from about 60 mM to about 80 mM, from about 80 mM to about 2 M, from about 80 mM to about 1 M, from about 80 mM to about 900 mM, from about 80 mM to about 800 mM, from about 80 mM to about 700 mM, from about 80 mM to about 600 mM,

15        from about 80 mM to about 500 mM, from about 80 mM to about 400 mM, from about 80 mM to about 300 mM, from about 80 mM to about 250 mM, from about 80 mM to about 200 mM, from about 80 mM to about 150 mM, from about 80 mM to about 100 mM, from about 100 mM to about 2 M, from about 100 mM to about 1 M, from about 100 mM to about 900 mM, from about 100 mM to about 800 mM, from about 100 mM to about 700

20        mM, from about 100 mM to about 600 mM, from about 100 mM to about 500 mM, from about 100 mM to about 400 mM, from about 100 mM to about 300 mM, from about 100 mM to about 250 mM, from about 100 mM to about 200 mM, from about 100 mM to about 150 mM, from about 150 mM to about 2 M, from about 150 mM to about 1 M, from about 150 mM to about 900 mM, from about 150 mM to about 800 mM, from about 150 mM to

25        about 700 mM, from about 150 mM to about 600 mM, from about 150 mM to about 500 mM, from about 150 mM to about 400 mM, from about 150 mM to about 300 mM, from about 150 mM to about 250 mM, from about 150 mM to about 200 mM, from about 200 mM to about 2 M, from about 200 mM to about 1 M, from about 200 mM to about 900 mM, from about 200 mM to about 800 mM, from about 200 mM to about 700 mM, from

30        about 200 mM to about 600 mM, from about 200 mM to about 500 mM, from about 200 mM to about 400 mM, from about 200 mM to about 300 mM, from about 200 mM to about 250 mM, from about 250 mM to about 2 M, from about 250 mM to about 1 M, from about 250 mM to about 900 mM, from about 250 mM to about 800 mM, from about 250 mM to

about 700 mM, from about 250 mM to about 600 mM, from about 250 mM to about 500 mM, from about 250 mM to about 400 mM, from about 250 mM to about 300 mM, from about 300 mM to about 2 M, from about 300 mM to about 1 M, from about 300 mM to about 900 mM, from about 300 mM to about 800 mM, from about 300 mM to about 700

5     mM, from about 300 mM to about 600 mM, from about 300 mM to about 500 mM, from about 300 mM to about 400 mM, from about 400 mM to about 2 M, from about 400 mM to about 1 M, from about 400 mM to about 900 mM, from about 400 mM to about 800 mM, from about 400 mM to about 700 mM, from about 400 mM to about 600 mM, from about 400 mM to about 500 mM, from about 500 mM to about 2 M, from about 500 mM to about

10    1 M, from about 500 mM to about 900 mM, from about 500 mM to about 800 mM, from about 500 mM to about 700 mM, from about 500 mM to about 600 mM, from about 600 mM to about 2 M, from about 600 mM to about 1 M, from about 600 mM to about 900 mM, from about 600 mM to about 800 mM, from about 600 mM to about 700 mM, from about 700 mM to about 2 M, from about 700 mM to about 1 M, from about 700 mM to

15    about 900 mM, from about 700 mM to about 800 mM, from about 800 mM to about 2 M, from about 800 mM to about 1 M, from about 800 mM to about 900 mM, from about 900 mM to about 2 M, from about 900 mM to about 1 M, from about 1 M to about 2 M, about 2 M, about 1 M, about 900 mM, about 800 mM, about 700 mM, about 600 mM, about 500 mM, about 400 mM, about 300 mM, about 250 mM, about 200 mM, about 150 mM, about

20    100 mM, about 80 mM, about 60 mM, about 50 mM, about 40 mM, about 30 mM, about 20 mM, about 10 mM, about 9 mM, about 8, mM, about 7 mM, about 6 mM, about 5 mM, about 4 mM, about 3 mM, about 2 mM, about 1 mM, 2 M, 1 M, 900 mM, 800 mM, 700 mM, 600 mM, 500 mM, 400 mM, 300 mM, 250 mM, 200 mM, 150 mM, 100 mM, 80 mM, 60 mM, 50 mM, 40 mM, 30 mM, 20 mM, 10 mM, 9 mM, 8, mM, 7 mM, 6 mM, 5 mM, 4

25    mM, 3 mM, 2 mM, or 1 mM.

The disclosed method of repairing and amplifying DNA can result in an increase in the average length of DNA fragment in a DNA sample. This increase can be referred to in any suitable terms. For example, the increase in average fragment length can be referred to by the average fragment length of the replicated DNA fragments, the increase in average

30    fragment length from the average fragment length of the damaged DNA sample, and the percent increase in average fragment length. The increase in average fragment length can be, for example, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 180%, 200%, 220%, 240%, 260%, 280%, 300%,

350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, 1,000%, 5% or more, 10% or more, 15% or more, 20% or more, 25% or more, 30% or more, 40% or more, 50% or more, 60% or more, 70% or more, 80% or more, 90% or more, 100% or more, 110% or more, 120% or more, 130% or more, 140% or more, 150% or more, 160% or more, 180% or more, 200% or more, 220% or more, 240% or more, 260% or more, 280% or more, 300% or more, 350% or more, 400% or more, 450% or more, 500% or more, 600% or more, 700% or more, 800% or more, 900% or more, 1,000% or more, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 110%, about 120%, about 130%, about 140%, about 150%, about 160%, about 180%, about 200%, about 220%, about 240%, about 260%, about 280%, about 300%, about 350%, about 400%, about 450%, about 500%, about 600%, about 700%, about 800%, about 900%, about 1,000%, about 5% or more, about 10% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 70% or more, about 80% or more, about 90% or more, about 100% or more, about 110% or more, about 120% or more, about 130% or more, about 140% or more, about 150% or more, about 160% or more, about 180% or more, about 200% or more, about 220% or more, about 240% or more, about 260% or more, about 280% or more, about 300% or more, about 350% or more, about 400% or more, about 450% or more, about 500% or more, about 600% or more, about 700% or more, about 800% or more, about 900% or more, or about 1,000% or more relative to the average fragment length of the damaged DNA sample before the method.

Following the repair method, the average fragment length can be, for example, 2 kilobases (kb), 2.5 kb, 3 kb, 3.5 kb, 4 kb, 4.5 kb, 5 kb, 5.5 kb, 6 kb, 7 kb, 8 kb, 9 kb, 10 kb, 11 kb, 12 kb, 13 kb, 14 kb, 15 kb, 16 kb, 18 kb, 20 kb, 22 kb, 24 kb, 26 kb, 28 kb, 30 kb, 2 kb or more, 2.5 kb or more, 3 kb or more, 3.5 kb or more, 4 kb or more, 4.5 kb or more, 5 kb or more, 5.5 kb or more, 6 kb or more, 7 kb or more, 8 kb or more, 9 kb or more, 10 kb or more, 11 kb or more, 12 kb or more, 13 kb or more, 14 kb or more, 15 kb or more, 16 kb or more, 18 kb or more, 20 kb or more, 22 kb or more, 24 kb or more, 26 kb or more, 28 kb or more, 30 kb or more, about 2 kb, about 2.5 kb, about 3 kb, about 3.5 kb, about 4 kb, about 4.5 kb, about 5 kb, about 5.5 kb, about 6 kb, about 7 kb, about 8 kb, about 9 kb, about 10 kb, about 11 kb, about 12 kb, about 13 kb, about 14 kb, about 15 kb, about 16 kb, about 18 kb, about 20 kb, about 22 kb, about 24 kb, about 26 kb, about 28 kb, about 30 kb, about 2 kb or more, about 2.5 kb or more, about 3 kb or more, about 3.5 kb or more, about 4 kb or

more, about 4.5 kb or more, about 5 kb or more, about 5.5 kb or more, about 6 kb or more, about 7 kb or more, about 8 kb or more, about 9 kb or more, about 10 kb or more, about 11 kb or more, about 12 kb or more, about 13 kb or more, about 14 kb or more, about 15 kb or more, about 16 kb or more, about 18 kb or more, about 20 kb or more, about 22 kb or more, about 24 kb or more, about 26 kb or more, about 28 kb or more, or about 30 kb or more.

The disclosed method of amplifying damaged DNA can be combined with the disclosed amplification of cell lysates and samples. Thus, for example, the damaged DNA samples can be a cell lysate or sample, where the cell lysate or sample is produced by exposing cells or the sample to alkaline conditions. Some forms of the method can include exposing cells to alkaline conditions to form a cell lysate; exposing the cell lysate to conditions that promote substantial denaturation of damaged DNA in the cell lysate; reducing the pH of the cell lysate to form a stabilized cell lysate; cooling the stabilized cell lysate under conditions that promote annealing of the ends of the denatured damaged DNA; and incubating the stabilized cell lysate under conditions that promote replication of the damaged DNA. During replication, the annealed ends of the damaged DNA prime replication and replication of the damaged DNA results in repair of the replicated strands and an increase in the average length of DNA fragment. The cell lysate or sample can be a whole genome. Replication of the genome results in replicated strands, where during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

In another form, the method works by hybridizing the ends of some DNA molecules in a sample to complementary sequences in a damaged DNA sample. Generally, the damaged DNA sample and the DNA sample providing the annealed ends or from the same source or even the same sample. Because the DNA molecules providing the newly associated ends and damaged DNA molecules will have damage at different locations, priming from the annealed ends can result in replication of more complete fragments and can mediate repair of the damaged DNA (in the form of less damaged or undamaged replicated strands). Replication of the undamaged replicated strands by continued multiple displacement amplification produces less damaged or undamaged amplified nucleic acids.

The method generally involves substantially denaturing a damaged DNA sample (generally via exposure to heat and alkaline conditions), reduction of the pH of the denatured DNA sample, mixing the denatured DNA sample with an undenatured DNA sample from the same source such that the ends of DNA in the undenatured DNA sample is

154

transiently denatured, slowly cooling the mixture of DNA samples to allow the transiently denatured ends to anneal to the denatured DNA, and replicating the annealed DNA. The damaged DNA is repaired during replication. The replication can be multiple displacement amplification. Substantial denaturation and transient denaturation of the DNA samples

5      generally is carried out such that the DNA is not further damaged. This method can generally be combined or used with any of the disclosed amplification methods.

In some embodiments, the method of amplifying damaged DNA can involve exposing a first damaged DNA sample to conditions that promote substantial denaturation of damaged DNA in the first damaged DNA sample, thereby forming a denatured damaged

10     DNA sample; reducing the pH of the denatured damaged DNA sample to form a stabilized denatured damaged DNA sample; mixing a second damaged DNA sample with the stabilized denatured damaged DNA sample under conditions that promote transient denaturation of the ends of damaged DNA in the second sample and that maintain substantial denaturation of the damaged DNA in the stabilized denatured damaged DNA

15     sample, thereby forming a damaged DNA mixture; cooling the damaged DNA mixture under conditions that promote annealing of the ends of the transiently denatured damaged DNA to the substantially denatured damaged DNA; and incubating the annealed damaged DNA under conditions that promote replication of the damaged DNA. The annealed ends of the damaged DNA prime replication and replication of the damaged DNA results in

20     repair of the replicated strands.

In the method, the first damaged DNA sample can be exposed to conditions that promote substantial denaturation by, for example, mixing the first damaged DNA sample with a denaturing solution and by heating the first damaged DNA sample to a temperature and for a length of time that substantially denatures the damaged DNA in the first damaged

25     DNA sample. The temperature can be, for example, about 25°C to about 50°C and the length of time can be, for example, about 5 minutes or more. The pH of the denatured damaged DNA sample can be reduced, for example, by mixing the denatured damaged DNA sample with a stabilization solution. The damaged DNA samples can be, for example, degraded DNA fragments of genomic DNA. The first and second damaged DNA samples

30     can be from the same source, and in particular can be a portion of the same damaged DNA sample. The second damaged DNA sample can be mixed with the stabilized denatured damaged DNA sample at a temperature and for a length of time that transiently denatures the damaged DNA in the second damaged DNA sample. For example, the temperature can

155

be about 70°C or less and the length of time can be about 30 seconds or less. The desired effect can also be achieved by maintaining the mixture at the temperature to which the first damaged DNA sample is exposed for denaturation. Replication and repair of the damaged DNA can be accomplished by incubating the annealed damaged DNA in the presence of a

5    DNA polymerase, such as φ29 DNA polymerase.

The disclosed method of amplifying damaged DNA can be combined with the disclosed amplification of cell lysates and samples. Thus, for example, the first and second damaged DNA samples can be portions of a cell lysate or sample, where the cell lysate or sample is produced by exposing cells or the sample to alkaline conditions. The pH of the

10   second damaged DNA sample can be reduced prior to mixing with the stabilized denatured damaged DNA. Some forms of the method can include exposing cells to alkaline conditions to form a cell lysate; exposing a first portion of the cell lysate to conditions that promote substantial denaturation of damaged DNA in the first portion of the cell lysate; reducing the pH of the first portion of the cell lysate to form a first stabilized cell lysate and

15   reducing the pH of a second portion of the cell lysate to form a second stabilized cell lysate; mixing the second stabilized cell lysate with the first stabilized cell lysate under conditions that promote transient denaturation of the ends of damaged DNA in the second stabilized cell lysate and that maintain substantial denaturation of the damaged DNA in the first stabilized cell lysate, thereby forming a stabilized cell lysate mixture; cooling the stabilized

20   cell lysate mixture under conditions that promote annealing of the ends of the transiently denatured damaged DNA to the substantially denatured damaged DNA; and incubating the stabilized cell lysate mixture under conditions that promote replication of the damaged DNA. During replication, the annealed ends of the damaged DNA prime replication and replication of the damaged DNA results in repair of the replicated strands. The cell lysate

25   or sample can be a whole genome. Replication of the genome results in replicated strands, where during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

## G. Analysis of Amplification Products

Clinical and health science studies require ready access to large quantities of

30   genomic DNA to serve as inputs for multiparametric assays of polymorphic sites in DNA, whose combined results provide valuable prognostic and diagnostic information. However, these studies are hampered by severe lack of adequate supply of DNA, as most biopsy methods yield only minute quantities of tissue or cells. Sample preparative steps further

156

reduce the amounts recovered from these cells due to loss during cell fractionation, thereby limiting the number of chromosomal loci that can be examined per sample using the isolated genomic DNA as input. Methods of the present invention seek to overcome these shortages by providing adequate and renewable supply of DNA for the multiparametric

5    analyses.

Analysis of loss of heterozygosity (LOH), a relatively common type of genetic alteration found throughout the genome in most solid neoplasms, is frequently employed in cancer diagnosis. While a number of familial cancer genes with high-penetrance mutations are readily identified, success in determining clinical outcomes by LOH analysis to evaluate

10   risk of sporadic cancer development is predicated also on contributions from low-penetrance genetic variants or polymorphisms. Such multiparametric assays require simultaneous analysis of a large number of candidate and other genetic loci from each sample for effective determination and statistical evaluation of disease progression and staging that are presently beyond the scope of measurements using native DNA prepared

15   from the clinical sample. Amplification of genomic DNA present in these samples is a useful adjunct for providing the necessary amounts of DNA required for the multiparametric analyses. The disclosed methods can provide high quality nucleic acids that provides sufficient material for analyses such as LOH analyses.

The progressive loss of form and structure of DNA in cancer cells culminates in

20   dozens of different genes becoming aberrant in nucleotide sequence or copy number, with hundreds or thousands of genes being differentially expressed in diseased cells compared to normal or premalignant cells. Elucidating the temporal and spatial attributes of the complex somatic genetic events delineating emerging cancer cells will aid the search for the more elusive germline variants that confer increased susceptibility. The disclosed methods can

25   provide sufficient amounts of nucleic acids amplified from sample sources to analyze these extensive changes in the genome. This can allow increased throughput of such measurements and efficient utilization of DNA recovered from these samples.

Some forms of the disclosed methods provide accurate and reproducible replication of sample DNA, so as to generate minimal, if any, changes in nucleotide sequence

30   distributions of replicated DNA strands from that of the input DNA. Many prior nucleic acid amplification methods introduce at least some significant degree of artifactual variation in sequence of the amplified DNA leading to bias in the representation of different

157

sequences in the amplified nucleic acids relative to representation of those sequences in the starting nucleic acids. Such bias can be referred to as sequence bias or allele bias.

In some cases, allele bias can be attributed to the properties of the polymerase enzyme employed in the amplification reaction. For example, 'proofreading ' DNA

5    polymerases are less susceptible to introducing allele bias in replicated DNA than DNA polymerases that lack proofreading activity. Misincorporation of one or more nucleotides by DNA polymerase during DNA synthesis could lead to replication bias or 'allele bias' during DNA amplification the change produces a different sequence that may be scored or detected as a different sequence or allele. Other factors that can contribute to replication

10   bias include the extent of or fold amplification of input DNA wherein more rounds of amplification could lead to increased allele bias in the replicated DNA, reaction conditions requiring treatment of amplification mixtures at elevated temperatures, treatments that promote creation of abasic sites in DNA, impurities in input DNA that may render the polymerase more error-prone, the nature and concentration of reagent components in the

15   amplification reaction, including presence of chaotropic agents, positively charged metal ions, and so on.

In some cases, nucleotide incorporation errors leading to allele bias can be due to a property of the nucleic acid template being amplified. For example, regions of DNA containing repeats or stretches of repeats of a single or few nucleotides can sometimes lead

20   to polymerase slippage, resulting in artifactual insertions or deletions of one or more nucleotides. For this reason, faithful amplification of DNA in repeat regions can be difficult to achieve. These regions include, di-, tri-, and tetra-nucleotide repeats, telomeric regions, regions containing long interspersed repeats, STR's and other kinds of repeats described in the literature. Regions of DNA containing extensive secondary structure can sometimes

25   prevent traverse of polymerase across the region, and may result in such sequences being under-represented among the replicated strand populations as well as introduction of allele bias.

Some forms of the disclosed methods provide for minimal differences in allele ratios between input nucleic acids and amplification products (which is allele bias--a form of

30   amplification bias). Allele ratio can be defined as the peak height (that is, amount detected) of the smaller allele divided by that of the larger allele (Paulson et al, 1999; herein incorporated by reference). Typically, allele ratios of a sample set of selected genetic loci are measured by performing genotyping assays of replicated or input DNA using a standard

genotyping assay. Genotyping assays are well known to one of ordinary skill in the art examples of which are described in US Patent Nos.5,451,067, 6,679,242, 6,479,244, 6,472,185, 6,458,544, 6,440,707, and 6,312,902, which are herein incorporated by reference. If the alleles are present in equal numbers (as would be expected for a

5    heterozygous locus), the allele ratio is 1 and there is no allele bias. As used herein, allele bias refers to a difference in the allele ratio for a pair of alleles from an allele ratio of 1. For alleles that do not have an even ratio (that is, a ratio of 1), allele bias can refer to a difference from the normal or expected allele ratio. Generally, the allele ratio for a locus in a heterozygous diploid sample will be 1, and this should be the allele ratio measurable in the

10   unamplified sample. When a sample is amplified, uneven amplification can result in a bias in the allele ratio. Allele bias can be calculated, for example, as the difference between the allele ratio of alleles in an unamplified sample and the allele ratio for the same alleles in DNA amplified from the sample. This can be referred to as amplification allele bias. Amplification allele bias, when present, indicates that the ratio of alleles in the amplified

15   DNA has been altered from the ratio in the original, unamplified genomic DNA. As an example, the allele ratio of two alleles of a locus that are present in equal number is 1. If the amplified DNA has a ratio of these two alleles of 0.5, then the amplification allele bias is 0.5 (calculated as 0.5/1 = 0.5). Such a bias can also be represented as 50% (referring to the difference in the ratios--0.5 is 50% of 1) or 2-fold (referring to the fold difference in the

20   allele ratios--1 is twice as large as 0.5).

Allele bias can also be quantified by assessing allele representation. The fraction of all alleles that a given allele represents is the allele representation for that allele. In the case where two alleles at a locus each represent half of the total (the normal case for heterozygous loci), then each allele can be said to have an allele representation of 50% or

25   0.5. Allele bias would be present if either allele had a representation different from 50%. If there is no difference between the allele representation in the input nucleic acid and the allele representation in the amplified DNA, then there is no allele bias, which can be represented as an allele bias of 1 or of 100%. In the case of allele representation, allele bias can be calculated as the ratio of the allele representations in two samples to be compared

30   (for example, unamplified sample versus amplified DNA). Thus, 50% representation over 50% representation equals 1. Allele bias can also be expressed as the standard deviation from an allele representation of 50% (or from the normal or expected allele representation).

When allele ratios of input and amplified DNA samples are same, then the amplified DNA is said to have no allele bias.

The disclosed method can accurately and evenly amplify the various sequences in highly complex nucleic acid samples. This result can be quantified by reference to, for example, percent representation, sequence representation, sequence representation bias, percent sequence representation, locus representation, locus representation bias, percent locus representation, and/or amplification bias. For example, the replicated nucleic acid molecules produced in the disclosed method can have a sequence representation or sequence representation bias of at least 50% for at least 10 different target sequences. The amplification bias can be less than 10% for at least 10 different target sequences.

The disclosed methods generally will produce amplified DNA with low allele bias. The disclosed methods can be used to measure allele bias and other amplification biases in amplified nucleic acids. For example, consider a case of an individual who is heterozygous for a selected genetic locus. The allele ratio of genomic DNA from this individual for this locus is one. An aliquot of genomic DNA from this individual could be subjected to whole genome amplification by employing the disclosed methods. If allele bias occurred during whole genome amplification, the amplified DNA would contain a greater representation of DNA copies of one of the parental alleles compared to the other parental allele. If DNA fragments containing either of the parental alleles greatly predominate in the amplified DNA population, then the genotyping test could score the DNA sample as being homozygous for that parental allele, leading to a misdiagnosis as homozygous normal. This failure to detect a heterozygous genotype as a consequence of nucleic acid amplification can be referred to as heterozygous dropout or allele drop out (ADO). In the case of homozygotes, wherein both parental alleles are same, ADO is easier to detect. The disclosed methods are equally adaptable to measuring ADO at homozygous loci.

It is unnecessary to assay for the presence of ADO at most or all of the loci present in amplified DNA. In fact, it would be impractical to do so. For most applications, assays of a sample of loci should suffice. Various factors can be considered for determining the number of loci to be scored in order to determine the ADO of an amplified DNA sample. These factors include, but are not limited to, the size (in nucleotides) of the genome being amplified, estimated error rates of nucleotide incorporation by the polymerase employed for amplification, amplification reaction conditions, and the duration of the amplification reaction. In general, a larger genome size is expected to produce larger values for ADO due

160

to the greater number of nucleotide additions per genome needed to complete amplification. The same is true of the duration of the amplification reaction, since a longer incubation time provides for a greater number of rounds of DNA amplification, thereby increasing the number of nucleotide additions. For instance, nucleotide misincorporation events that occur

5      during early rounds of replication are more likely to be perpetuated and predominate in the final product of the amplification reaction, than those occurring during later rounds of amplification. For purposes of this calculation, contributions from reversions of misincorporated nucleotides to wild type are ignored, since these events are of very low probability, except at mutational hotspots. Different polymerases vary greatly in their rates

10     of nucleotide incorporation errors generated during DNA replication. This is due, in part, to an intrinsic property of the polymerase itself. In general, polymerases lacking a 3', 5'-exonuclease activity are more error-prone than those that possess such activity. Other factors that contribute to misincorporation of nucleotides by polymerases are known, and include, for example, the presence of impurities in the amplification reaction and the

15     presence of reagents that alter the structure of the polymerase or otherwise render them error prone, including organic reagents and divalent metal ions. In general, the number of loci to be evaluated in the amplified DNA to obtain an estimate of the ADO can be estimated by the equation:

$$(G * RC)/PER$$

20     where G is the size of the genome (or complexity of the nucleic acids), RC is the average number of rounds of replication in the amplification, and PER is the polymerase error rate (that is, the rate of misincorporation of nucleotides).

In preferred embodiments, ADO can be determined by scoring alleles at only a sample number of loci. Typically, 2-8% of the number of ADO sites estimated using the

25     equation above can be assayed. In general, 100-500 loci can be selected when human genomic DNA is employed in the amplification reaction. Selection of loci for ADO assays can be random or ordered. In preferred embodiments, loci can be selected on the basis of their location on a chromosome of interest, in close proximity to or greater than a selected genetic distance away from a locus or chromosomal landmark of interest, on the basis of

30     known loci that are hypersensitive to ADO, or other criteria. An ordered selection of loci can further reduce the number of loci that need to be evaluated for measuring ADO. Thus, results from assays involving 1 to 2 loci, 2 to 5 loci, 5 to 10 loci, 10 to 20 loci, 20 to 50 loci,

50 to 100 loci, 100 to 200 loci, 200 to 500 loci , or more than 500 loci may suffice for measuring ADO.

## H. Amplified Nucleic Acid Quality

The disclosed method can result in replication of all or a substantial fraction of the nucleic acid molecules in a nucleic acid sample. As used herein, a substantial fraction of the nucleic acid molecules in a nucleic acid sample refers to 90% or more of the nucleic acid molecules (or nucleic acid sequences) present in the nucleic acid sample. As used herein, a significant fraction of the nucleic acid molecules in a nucleic acid sample refers to 50% or more of the nucleic acid molecules (or nucleic acid sequences) present in the nucleic acid sample. As used herein, a notable fraction of the nucleic acid molecules in a nucleic acid sample refers to 20% or more of the nucleic acid molecules (or nucleic acid sequences) present in the nucleic acid sample.

Replication of the nucleic acid molecules in a nucleic acid sample can result replication of at least 0.01% of the nucleic acid sequences in the nucleic acid sample, at least 0.1% of the nucleic acid sequences in the nucleic acid sample, at least 1% of the nucleic acid sequences in the nucleic acid sample, at least 5% of the nucleic acid sequences in the nucleic acid sample, at least 10% of the nucleic acid sequences in the nucleic acid sample, at least 20% of the nucleic acid sequences in the nucleic acid sample, at least 30% of the nucleic acid sequences in the nucleic acid sample, at least 40% of the nucleic acid sequences in the nucleic acid sample, at least 50% of the nucleic acid sequences in the nucleic acid sample, at least 60% of the nucleic acid sequences in the nucleic acid sample, at least 70% of the nucleic acid sequences in the nucleic acid sample, at least 80% of the nucleic acid sequences in the nucleic acid sample, at least 90% of the nucleic acid sequences in the nucleic acid sample, at least 95% of the nucleic acid sequences in the nucleic acid sample, at least 96% of the nucleic acid sequences in the nucleic acid sample, at least 97% of the nucleic acid sequences in the nucleic acid sample, at least 98% of the nucleic acid sequences in the nucleic acid sample, or at least 99% of the nucleic acid sequences in the nucleic acid sample.

The fraction of the nucleic acid molecules in the nucleic acid sample that is replicated can vary with the sequence complexity of the nucleic acid sample (although higher fractions are preferred for all nucleic acid samples). For example, where the nucleic acid sample has a sequence complexity of at least $1 \times 10^9$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 0.01% of the

nucleic acid sequences in the nucleic acid sample.  Where the nucleic acid sample has a sequence complexity of at least $1 \times 10^8$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 0.1% of the nucleic acid sequences in the nucleic acid sample.  Where the nucleic acid sample has a sequence complexity of at least $1 \times 10^7$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 1% of the nucleic acid sequences in the nucleic acid sample.  Where the nucleic acid sample has a sequence complexity of at least $1 \times 10^6$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 10% of the nucleic acid sequences in the nucleic acid sample.  Where the nucleic acid sample has a sequence complexity of at least $1 \times 10^5$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 80% of the nucleic acid sequences in the nucleic acid sample.  Where the nucleic acid sample has a sequence complexity of at least $1 \times 10^4$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 90% of the nucleic acid sequences in the nucleic acid sample.  Where the nucleic acid sample has a sequence complexity of at least $1 \times 10^3$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 96% of the nucleic acid sequences in the nucleic acid sample.

Where the nucleic acid sample has a sequence complexity of less than $1 \times 10^9$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 0.01% of the nucleic acid sequences in the nucleic acid sample.  Where the nucleic acid sample has a sequence complexity of less than $1 \times 10^8$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 0.1% of the nucleic acid sequences in the nucleic acid sample.  Where the nucleic acid sample has a sequence complexity of less than $1 \times 10^7$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 1% of the nucleic acid sequences in the nucleic acid sample.  Where the nucleic acid sample has a sequence complexity of less than $1 \times 10^6$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 10% of the nucleic acid sequences in the nucleic acid sample.  Where the nucleic acid sample has a sequence complexity of less than $1 \times 10^5$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 80% of the nucleic acid sequences in the nucleic acid sample.  Where the nucleic acid sample has a sequence complexity of less

163

than $1 \times 10^4$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in replication of at least 90% of the nucleic acid sequences in the nucleic acid sample. Where the nucleic acid sample has a sequence complexity of less than $1 \times 10^3$ nucleotides, replication of nucleic acid molecules in the nucleic acid sample can result in

5        replication of at least 96% of the nucleic acid sequences in the nucleic acid sample.

One measure of the quality of the amplified nucleic acids can be the percent representation, sequence representation, sequence representation bias, percent sequence representation, locus representation, locus representation bias, and/or percent locus representation in the amplified nucleic acids. A locus representation or sequence

10       representation the same as or close to the locus or sequence representation in the source nucleic acid sample indicates amplified nucleic acids of the highest quality. Locus representation bias can refer to the ratio (usually expressed as a percentage) of the amount of a given locus in amplified nucleic acid to the amount of the same locus in the unamplified nucleic acid sample. In making this calculation, the measured amount of the

15       locus in the amplified nucleic and the measured amount of the locus in the unamplified nucleic acid sample generally can be normalized to the total amount of nucleic acid present in the amplified nucleic acid and the unamplified nucleic acid sample, respectively. Locus representation or locus representation bias expressed as a percentage (usually of a reference locus representation) can be referred to as a percent locus representation (which is a form of

20       percent representation). Locus representation bias can also be expressed as the standard deviation of the locus representation in an amplified sample from the locus representation in the unamplified sample (or other reference locus representation). Locus representation bias can be a form of amplification bias. Locus representation can refer to the amount or level of a given locus (or a group of loci). Locus representation can be expressed as a locus

25       representation relative to another, reference locus representation. Thus, for example, a percent locus representation is a form of locus representation.

Sequence representation bias can refer to the ratio (usually expressed as a percentage) of the amount of a given sequence in amplified nucleic acid to the amount of the same sequence in the unamplified nucleic acid sample. In making this calculation, the

30       measured amount of the sequence in the amplified nucleic and the measured amount of the sequence in the unamplified nucleic acid sample generally can be normalized to the total amount of nucleic acid present in the amplified nucleic acid and the unamplified nucleic acid sample, respectively. Sequence representation or sequence representation bias

expressed as a percentage (usually of a reference sequence representation) can be referred to as a percent sequence representation (which is a form of percent representation). Sequence representation bias can also be expressed as the standard deviation of the sequence representation in an amplified sample from the sequence representation in the unamplified

5    sample (or other reference locus representation). Sequence representation bias can be a form of amplification bias. Sequence representation can refer to the amount or level of a given sequence (or a group of sequences). Sequence representation can be expressed as a sequence representation relative to another, reference sequence representation. Thus, for example, a percent sequence representation is a form of sequence representation.

10        The locus or sequence representation or locus or sequence representation bias can be, for example, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 225%, 250%, 275%, 300%, 350%, 400%, 450%, 500%, 600%, 700%, 800%, 900%, or 1000% for one, some, or all loci or sequences measured. The locus or sequence representation or locus or sequence

15   representation bias can be, for example, greater than 10%, greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 100%, greater than 110%, greater than 120%, greater than 130%, greater than 140%, greater than 150%, greater than 160%, greater than 170%, greater than 180%, greater than 190%, greater than 200%, greater than 225%, greater than

20   250%, greater than 275%, greater than 300%, greater than 350%, greater than 400%, greater than 450%, greater than 500%, greater than 600%, greater than 700%, greater than 800%, greater than 900%, or greater than 1000% for one, some, or all loci or sequences measured. The locus or sequence representation or locus or sequence representation bias can be, for example, less than 10%, less than 20%, less than 30%, less than 40%, less than 50%, less

25   than 60%, less than 70%, less than 80%, less than 90%, less than 100%, less than 110%, less than 120%, less than 130%, less than 140%, less than 150%, less than 160%, less than 170%, less than 180%, less than 190%, less than 200%, less than 225%, less than 250%, less than 275%, less than 300%, less than 350%, less than 400%, less than 450%, less than 500%, less than 600%, less than 700%, less than 800%, less than 900%, or less than 1000%

30   for one, some, or all loci or sequences measured.

        The locus or sequence representation or locus or sequence representation bias can be, for example, between 10% and 1000%, between 10% and 900%, between 10% and 800%, between 10% and 700%, between 10% and 600%, between 10% and 500%, between

10% and 400%, between 10% and 300%, between 10% and 250%, between 10% and 200%, between 10% and 150%, between 10% and 125%, between 10% and 100%, between 20% and 1000%, between 20% and 900%, between 20% and 800%, between 20% and 700%, between 20% and 600%, between 20% and 500%, between 20% and 400%, between 20% and 300%, between 20% and 250%, between 20% and 200%, between 20% and 150%, between 20% and 125%, between 20% and 100%, between 30% and 1000%, between 30% and 900%, between 30% and 800%, between 30% and 700%, between 30% and 600%, between 30% and 500%, between 30% and 400%, between 30% and 300%, between 30% and 250%, between 30% and 200%, between 30% and 150%, between 30% and 125%, between 30% and 100%, between 40% and 1000%, between 40% and 900%, between 40% and 800%, between 40% and 700%, between 40% and 600%, between 40% and 500%, between 40% and 400%, between 40% and 300%, between 40% and 250%, between 40% and 200%, between 40% and 150%, between 40% and 125%, between 40% and 100%, between 50% and 1000%, between 50% and 900%, between 50% and 800%, between 50% and 700%, between 50% and 600%, between 50% and 500%, between 50% and 400%, between 50% and 300%, between 50% and 250%, between 50% and 200%, between 50% and 150%, between 50% and 125%, between 50% and 100%, between 60% and 1000%, between 60% and 900%, between 60% and 800%, between 60% and 700%, between 60% and 600%, between 60% and 500%, between 60% and 400%, between 60% and 300%, between 60% and 250%, between 60% and 200%, between 60% and 150%, between 60% and 125%, between 60% and 100%, between 70% and 1000%, between 70% and 900%, between 70% and 800%, between 70% and 700%, between 70% and 600%, between 70% and 500%, between 70% and 400%, between 70% and 300%, between 70% and 250%, between 70% and 200%, between 70% and 150%, between 70% and 125%, between 70% and 100%, between 80% and 1000%, between 80% and 900%, between 80% and 800%, between 80% and 700%, between 80% and 600%, between 80% and 500%, between 80% and 400%, between 80% and 300%, between 80% and 250%, between 80% and 200%, between 80% and 150%, between 80% and 125%, between 80% and 100%, between 90% and 1000%, between 90% and 900%, between 90% and 800%, between 90% and 700%, between 90% and 600%, between 90% and 500%, between 90% and 400%, between 90% and 300%, between 90% and 250%, between 90% and 200%, between 90% and 150%, between 90% and 125%, between 90% and 100%, between 100% and 1000%, between 100% and 900%, between 100% and 800%, between 100% and 700%, between 100% and

600%, between 100% and 500%, between 100% and 400%, between 100% and 300%, between 100% and 250%, between 100% and 200%, between 100% and 150%, or between 100% and 125% for one, some, or all loci or sequences measured.

The various locus representations and locus representation biases described above and elsewhere herein can be, for example, for 1 locus, 2 loci, 3 loci, 4 loci, 5 loci, 6 loci, 7 loci, 8 loci, 9 loci, 10 loci, 11 loci, 12 loci, 13 loci, 14 loci, 15 loci, 16 loci, 17 loci, 18 loci, 19 loci, 20 loci, 25 loci, 30 loci, 40 loci, 50 loci, 75 loci, or 100 loci. The locus representation or locus representation bias can be, for example, for at least 1 locus, at least 2 loci, at least 3 loci, at least 4 loci, at least 5 loci, at least 6 loci, at least 7 loci, at least 8 loci, at least 9 loci, at least 10 loci, at least 11 loci, at least 12 loci, at least 13 loci, at least 14 loci, at least 15 loci, at least 16 loci, at least 17 loci, at least 18 loci, at least 19 loci, at least 20 loci, at least 25 loci, at least 30 loci, at least 40 loci, at least 50 loci, at least 75 loci, or at least 100 loci.

The locus representation or locus representation bias can be, for example, for 1 locus, 2 different loci, 3 different loci, 4 different loci, 5 different loci, 6 different loci, 7 different loci, 8 different loci, 9 different loci, 10 different loci, 11 different loci, 12 different loci, 13 different loci, 14 different loci, 15 different loci, 16 different loci, 17 different loci, 18 different loci, 19 different loci, 20 different loci, 25 different loci, 30 different loci, 40 different loci, 50 different loci, 75 different loci, or 100 different loci. The locus representation or locus representation bias can be, for example, for at least 1 locus, at least 2 different loci, at least 3 different loci, at least 4 different loci, at least 5 different loci, at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

The various sequence representations and sequence representation biases described above and elsewhere herein can be, for example, for 1 target sequence, 2 target sequences, 3 target sequences, 4 target sequences, 5 target sequences, 6 target sequences, 7 target sequences, 8 target sequences, 9 target sequences, 10 target sequences, 11 target sequences, 12 target sequences, 13 target sequences, 14 target sequences, 15 target sequences, 16 target sequences, 17 target sequences, 18 target sequences, 19 target sequences, 20 target

sequences, 25 target sequences, 30 target sequences, 40 target sequences, 50 target sequences, 75 target sequences, or 100 target sequences. The sequence representation or sequence representation bias can be, for example, for at least 1 target sequence, at least 2 target sequences, at least 3 target sequences, at least 4 target sequences, at least 5 target

5   sequences, at least 6 target sequences, at least 7 target sequences, at least 8 target sequences, at least 9 target sequences, at least 10 target sequences, at least 11 target sequences, at least 12 target sequences, at least 13 target sequences, at least 14 target sequences, at least 15 target sequences, at least 16 target sequences, at least 17 target sequences, at least 18 target sequences, at least 19 target sequences, at least 20 target sequences, at least 25 target

10  sequences, at least 30 target sequences, at least 40 target sequences, at least 50 target sequences, at least 75 target sequences, or at least 100 target sequences.

The sequence representation or sequence representation bias can be, for example, for 1 target sequence, 2 different target sequences, 3 different target sequences, 4 different target sequences, 5 different target sequences, 6 different target sequences, 7 different target

15  sequences, 8 different target sequences, 9 different target sequences, 10 different target sequences, 11 different target sequences, 12 different target sequences, 13 different target sequences, 14 different target sequences, 15 different target sequences, 16 different target sequences, 17 different target sequences, 18 different target sequences, 19 different target sequences, 20 different target sequences, 25 different target sequences, 30 different target

20  sequences, 40 different target sequences, 50 different target sequences, 75 different target sequences, or 100 different target sequences. The sequence representation or sequence representation bias can be, for example, for at least 1 target sequence, at least 2 different target sequences, at least 3 different target sequences, at least 4 different target sequences, at least 5 different target sequences, at least 6 different target sequences, at least 7 different

25  target sequences, at least 8 different target sequences, at least 9 different target sequences, at least 10 different target sequences, at least 11 different target sequences, at least 12 different target sequences, at least 13 different target sequences, at least 14 different target sequences, at least 15 different target sequences, at least 16 different target sequences, at least 17 different target sequences, at least 18 different target sequences, at least 19 different target

30  sequences, at least 20 different target sequences, at least 25 different target sequences, at least 30 different target sequences, at least 40 different target sequences, at least 50 different target sequences, at least 75 different target sequences, or at least 100 different target sequences.

Another measure of the quality of the amplified nucleic acids can be the amplification bias in the amplified nucleic acids. Amplification bias is the difference in the level of amplification of different sequences in a nucleic acid sample. A low amplification bias indicates amplified nucleic acids of the highest quality. One expression of

5      amplification bias can be calculated as the ratio (usually expressed as a fold difference or a percent difference) of the locus representation bias of the locus having the highest locus representation bias to the locus representation bias having the lowest locus representation bias in the amplified nucleic acid. Another expression of amplification bias can be calculated as the ratio (usually expressed as a fold difference or a percent difference) of the

10     locus representation of the locus having the highest locus representation to the locus representation having the lowest locus representation in the amplified nucleic acid. If sequence representation bias is measured, then amplification bias can be calculated as the ratio (usually expressed as a fold difference) of the sequence representation bias of the sequence having the highest sequence representation bias to the sequence representation

15     bias having the lowest sequence representation bias in the amplified nucleic acid. Amplification bias can be calculated as the ratio (usually expressed as a fold difference) of the sequence representation of the sequence having the highest sequence representation to the sequence representation having the lowest sequence representation in the amplified nucleic acid. Although the above calculations are measures of amplification bias for all of

20     the loci or sequences assessed, a subset of loci or sequences assessed can be used to calculate amplification bias. In fact, amplification bias can be calculated for individual loci, sequences or alleles. Thus, for example, amplification bias can also be calculated as the ratio (usually expressed as a fold difference or a percent difference) of the locus representation bias of one or more loci to the locus representation bias one or more other

25     loci in the amplified nucleic acid. As another example, amplification bias can also be calculated as the ratio (usually expressed as a fold difference or a percent difference) of the locus representation in an unamplified sample of one or more loci to the locus representation in an amplified sample of the same loci.

The amplification bias can be, for example, 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-

30     fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 14-fold, 16-fold, 20-fold, 24-fold, 30-fold, 35-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, 200-fold, 250-fold, or 300-fold. The amplification bias can be, for example, about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold,

169

about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 14-fold, about 16-fold, about 20-fold, about 24-fold, about 30-fold, about 35-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, about 100-fold, about 150-fold, about 200-fold, about 250-fold, or about 300-fold. The amplification bias can be, for example, less than 2-fold, less than 3-fold, less than 4-fold, less than 5-fold, less than 6-fold, less than 7-fold, less than 8-fold, less than 9-fold, less than 10-fold, less than 11-fold, less than 12-fold, less than 14-fold, less than 16-fold, less than 20-fold, less than 24-fold, less than 30-fold, less than 35-fold, less than 40-fold, less than 50-fold, less than 60-fold, less than 70-fold, less than 80-fold, less than 90-fold, less than 100-fold, less than 150-fold, less than 200-fold, less than 250-fold, or less than 300-fold.

The amplification bias can be, for example, less than about 2-fold, less than about 3-fold, less than about 4-fold, less than about 5-fold, less than about 6-fold, less than about 7-fold, less than about 8-fold, less than about 9-fold, less than about 10-fold, less than about 11-fold, less than about 12-fold, less than about 14-fold, less than about 16-fold, less than about 20-fold, less than about 24-fold, less than about 30-fold, less than about 35-fold, less than about 40-fold, less than about 50-fold, less than about 60-fold, less than about 70-fold, less than about 80-fold, less than about 90-fold, less than about 100-fold, less than about 150-fold, less than about 200-fold, less than about 250-fold, or less than about 300-fold.

The amplification bias can be, for example, from 1-fold to 300-fold, from 2-fold to 300-fold, from 3-fold to 300-fold, from 4-fold to 300-fold, from 5-fold to 300-fold, from 6-fold to 300-fold, from 7-fold to 300-fold, from 8-fold to 300-fold, from 9-fold to 300-fold, from 10-fold to 300-fold, from 11-fold to 300-fold, from 12-fold to 300-fold, from 14-fold to 300-fold, from 16-fold to 300-fold, from 20-fold to 300-fold, from 24-fold to 300-fold, from 30-fold to 300-fold, from 35-fold to 300-fold, from 40-fold to 300-fold, from 50-fold to 300-fold, from 60-fold to 300-fold, from 70-fold to 300-fold, from 80-fold to 300-fold, from 90-fold to 300-fold, from 100-fold to 300-fold, from 150-fold to 300-fold, from 200-fold to 300-fold, or from 250-fold to 300-fold.

The amplification bias can be, for example, from 1-fold to 250-fold, from 2-fold to 250-fold, from 3-fold to 250-fold, from 4-fold to 250-fold, from 5-fold to 250-fold, from 6-fold to 250-fold, from 7-fold to 250-fold, from 8-fold to 250-fold, from 9-fold to 250-fold, from 10-fold to 250-fold, from 11-fold to 250-fold, from 12-fold to 250-fold, from 14-fold to 250-fold, from 16-fold to 250-fold, from 20-fold to 250-fold, from 24-fold to 250-fold, from 30-fold to 250-fold, from 35-fold to 250-fold, from 40-fold to 250-fold, from 50-fold

to 250-fold, from 60-fold to 250-fold, from 70-fold to 250-fold, from 80-fold to 250-fold, from 90-fold to 250-fold, from 100-fold to 250-fold, from 150-fold to 250-fold, or from 200-fold to 250-fold.

The amplification bias can be, for example, from 1-fold to 200-fold, from 2-fold to 200-fold, from 3-fold to 200-fold, from 4-fold to 200-fold, from 5-fold to 200-fold, from 6-fold to 200-fold, from 7-fold to 200-fold, from 8-fold to 200-fold, from 9-fold to 200-fold, from 10-fold to 200-fold, from 11-fold to 200-fold, from 12-fold to 200-fold, from 14-fold to 200-fold, from 16-fold to 200-fold, from 20-fold to 200-fold, from 24-fold to 200-fold, from 30-fold to 200-fold, from 35-fold to 200-fold, from 40-fold to 200-fold, from 50-fold to 200-fold, from 60-fold to 200-fold, from 70-fold to 200-fold, from 80-fold to 200-fold, from 90-fold to 200-fold, from 100-fold to 200-fold, or from 150-fold to 200-fold.

The amplification bias can be, for example, from 1-fold to 150-fold, from 2-fold to 150-fold, from 3-fold to 150-fold, from 4-fold to 150-fold, from 5-fold to 150-fold, from 6-fold to 150-fold, from 7-fold to 150-fold, from 8-fold to 150-fold, from 9-fold to 150-fold, from 10-fold to 150-fold, from 11-fold to 150-fold, from 12-fold to 150-fold, from 14-fold to 150-fold, from 16-fold to 150-fold, from 20-fold to 150-fold, from 24-fold to 150-fold, from 30-fold to 150-fold, from 35-fold to 150-fold, from 40-fold to 150-fold, from 50-fold to 150-fold, from 60-fold to 150-fold, from 70-fold to 150-fold, from 80-fold to 150-fold, from 90-fold to 150-fold, or from 100-fold to 150-fold.

The amplification bias can be, for example, from 1-fold to 100-fold, from 2-fold to 100-fold, from 3-fold to 100-fold, from 4-fold to 100-fold, from 5-fold to 100-fold, from 6-fold to 100-fold, from 7-fold to 100-fold, from 8-fold to 100-fold, from 9-fold to 100-fold, from 10-fold to 100-fold, from 11-fold to 100-fold, from 12-fold to 100-fold, from 14-fold to 100-fold, from 16-fold to 100-fold, from 20-fold to 100-fold, from 24-fold to 100-fold, from 30-fold to 100-fold, from 35-fold to 100-fold, from 40-fold to 100-fold, from 50-fold to 100-fold, from 60-fold to 100-fold, from 70-fold to 100-fold, from 80-fold to 100-fold, or from 90-fold to 100-fold.

The amplification bias can be, for example, from 1-fold to 90-fold, from 2-fold to 90-fold, from 3-fold to 90-fold, from 4-fold to 90-fold, from 5-fold to 90-fold, from 6-fold to 90-fold, from 7-fold to 90-fold, from 8-fold to 90-fold, from 9-fold to 90-fold, from 10-fold to 90-fold, from 11-fold to 90-fold, from 12-fold to 90-fold, from 14-fold to 90-fold, from 16-fold to 90-fold, from 20-fold to 90-fold, from 24-fold to 90-fold, from 30-fold to

90-fold, from 35-fold to 90-fold, from 40-fold to 90-fold, from 50-fold to 90-fold, from 60-fold to 90-fold, from 70-fold to 90-fold, or from 80-fold to 90-fold.

The amplification bias can be, for example, from 1-fold to 80-fold, from 2-fold to 80-fold, from 3-fold to 80-fold, from 4-fold to 80-fold, from 5-fold to 80-fold, from 6-fold to 80-fold, from 7-fold to 80-fold, from 8-fold to 80-fold, from 9-fold to 80-fold, from 10-fold to 80-fold, from 11-fold to 80-fold, from 12-fold to 80-fold, from 14-fold to 80-fold, from 16-fold to 80-fold, from 20-fold to 80-fold, from 24-fold to 80-fold, from 30-fold to 80-fold, from 35-fold to 80-fold, from 40-fold to 80-fold, from 50-fold to 80-fold, from 60-fold to 80-fold, or from 70-fold to 80-fold.

The amplification bias can be, for example, from 1-fold to 70-fold, from 2-fold to 70-fold, from 3-fold to 70-fold, from 4-fold to 70-fold, from 5-fold to 70-fold, from 6-fold to 70-fold, from 7-fold to 70-fold, from 8-fold to 70-fold, from 9-fold to 70-fold, from 10-fold to 70-fold, from 11-fold to 70-fold, from 12-fold to 70-fold, from 14-fold to 70-fold, from 16-fold to 70-fold, from 20-fold to 70-fold, from 24-fold to 70-fold, from 30-fold to 70-fold, from 35-fold to 70-fold, from 40-fold to 70-fold, from 50-fold to 70-fold, or from 60-fold to 70-fold. The amplification bias can be, for example, from 1-fold to 60-fold, from 2-fold to 60-fold, from 3-fold to 60-fold, from 4-fold to 60-fold, from 5-fold to 60-fold, from 6-fold to 60-fold, from 7-fold to 60-fold, from 8-fold to 60-fold, from 9-fold to 60-fold, from 10-fold to 60-fold, from 11-fold to 60-fold, from 12-fold to 60-fold, from 14-fold to 60-fold, from 16-fold to 60-fold, from 20-fold to 60-fold, from 24-fold to 60-fold, from 30-fold to 60-fold, from 35-fold to 60-fold, from 40-fold to 60-fold, or from 50-fold to 60-fold.

The amplification bias can be, for example, from 1-fold to 50-fold, from 2-fold to 50-fold, from 3-fold to 50-fold, from 4-fold to 50-fold, from 5-fold to 50-fold, from 6-fold to 50-fold, from 7-fold to 50-fold, from 8-fold to 50-fold, from 9-fold to 50-fold, from 10-fold to 50-fold, from 11-fold to 50-fold, from 12-fold to 50-fold, from 14-fold to 50-fold, from 16-fold to 50-fold, from 20-fold to 50-fold, from 24-fold to 50-fold, from 30-fold to 50-fold, from 35-fold to 50-fold, or from 40-fold to 50-fold. The amplification bias can be, for example, from 1-fold to 40-fold, from 2-fold to 40-fold, from 3-fold to 40-fold, from 4-fold to 40-fold, from 5-fold to 40-fold, from 6-fold to 40-fold, from 7-fold to 40-fold, from 8-fold to 40-fold, from 9-fold to 40-fold, from 10-fold to 40-fold, from 11-fold to 40-fold, from 12-fold to 40-fold, from 14-fold to 40-fold, from 16-fold to 40-fold, from 20-fold to 40-fold, from 24-fold to 40-fold, from 30-fold to 40-fold, or from 35-fold to 40-fold.

The amplification bias can be, for example, from 1-fold to 30-fold, from 2-fold to 30-fold, from 3-fold to 30-fold, from 4-fold to 30-fold, from 5-fold to 30-fold, from 6-fold to 30-fold, from 7-fold to 30-fold, from 8-fold to 30-fold, from 9-fold to 30-fold, from 10-fold to 30-fold, from 11-fold to 30-fold, from 12-fold to 30-fold, from 14-fold to 30-fold,

5      from 16-fold to 30-fold, from 20-fold to 30-fold, or from 24-fold to 30-fold.  The amplification bias can be, for example, from 1-fold to 20-fold, from 2-fold to 20-fold, from 3-fold to 20-fold, from 4-fold to 20-fold, from 5-fold to 20-fold, from 6-fold to 20-fold, from 7-fold to 20-fold, from 8-fold to 20-fold, from 9-fold to 20-fold, from 10-fold to 20-fold, from 11-fold to 20-fold, from 12-fold to 20-fold, from 14-fold to 20-fold, from 16-fold

10     to 20-fold, from 20-fold to 20-fold, or from 24-fold to 20-fold.

The amplification bias can be, for example, from 1-fold to 12-fold, from 2-fold to 12-fold, from 3-fold to 12-fold, from 4-fold to 12-fold, from 5-fold to 12-fold, from 6-fold to 12-fold, from 7-fold to 12-fold, from 8-fold to 12-fold, from 9-fold to 12-fold, from 10-fold to 12-fold, or from 11-fold to 12-fold.  The amplification bias can be, for example,

15     from 1-fold to 11-fold, from 2-fold to 11-fold, from 3-fold to 11-fold, from 4-fold to 11-fold, from 5-fold to 11-fold, from 6-fold to 11-fold, from 7-fold to 11-fold, from 8-fold to 11-fold, from 9-fold to 11-fold, or from 10-fold to 11-fold.  The amplification bias can be, for example, from 1-fold to 10-fold, from 2-fold to 10-fold, from 3-fold to 10-fold, from 4-fold to 10-fold, from 5-fold to 10-fold, from 6-fold to 10-fold, from 7-fold to 10-fold, from

20     8-fold to 10-fold, or from 9-fold to 10-fold.  The amplification bias can be, for example, from 1-fold to 9-fold, from 2-fold to 9-fold, from 3-fold to 9-fold, from 4-fold to 9-fold, from 5-fold to 9-fold, from 6-fold to 9-fold, from 7-fold to 9-fold, or from 8-fold to 9-fold.

The amplification bias can be, for example, from 1-fold to 8-fold, from 2-fold to 8-fold, from 3-fold to 8-fold, from 4-fold to 8-fold, from 5-fold to 8-fold, from 6-fold to 8-

25     fold, or from 7-fold to 8-fold.  The amplification bias can be, for example, from 1-fold to 7-fold, from 2-fold to 7-fold, from 3-fold to 7-fold, from 4-fold to 7-fold, from 5-fold to 7-fold, or from 6-fold to 7-fold.  The amplification bias can be, for example, from 1-fold to 6-fold, from 2-fold to 6-fold, from 3-fold to 6-fold, from 4-fold to 6-fold, or from 5-fold to 6-fold.  The amplification bias can be, for example, from 1-fold to 5-fold, from 2-fold to 5-

30     fold, from 3-fold to 5-fold, from 4-fold to 5-fold, from 1-fold to 4-fold, from 2-fold to 4-fold, from 3-fold to 4-fold, from 1-fold to 3-fold, from 2-fold to 3-fold, or from 1-fold to 2-fold.

The amplification bias can be, for example, from about 1-fold to about 300-fold, from about 2-fold to about 300-fold, from about 3-fold to about 300-fold, from about 4-fold to about 300-fold, from about 5-fold to about 300-fold, from about 6-fold to about 300-fold, from about 7-fold to about 300-fold, from about 8-fold to about 300-fold, from about 9-fold to about 300-fold, from about 10-fold to about 300-fold, from about 11-fold to about 300-fold, from about 12-fold to about 300-fold, from about 14-fold to about 300-fold, from about 16-fold to about 300-fold, from about 20-fold to about 300-fold, from about 24-fold to about 300-fold, from about 30-fold to about 300-fold, from about 35-fold to about 300-fold, from about 40-fold to about 300-fold, from about 50-fold to about 300-fold, from about 60-fold to about 300-fold, from about 70-fold to about 300-fold, from about 80-fold to about 300-fold, from about 90-fold to about 300-fold, from about 100-fold to about 300-fold, from about 150-fold to about 300-fold, from about 200-fold to about 300-fold, or from about 250-fold to about 300-fold.

The amplification bias can be, for example, from about 1-fold to about 250-fold, from about 2-fold to about 250-fold, from about 3-fold to about 250-fold, from about 4-fold to about 250-fold, from about 5-fold to about 250-fold, from about 6-fold to about 250-fold, from about 7-fold to about 250-fold, from about 8-fold to about 250-fold, from about 9-fold to about 250-fold, from about 10-fold to about 250-fold, from about 11-fold to about 250-fold, from about 12-fold to about 250-fold, from about 14-fold to about 250-fold, from about 16-fold to about 250-fold, from about 20-fold to about 250-fold, from about 24-fold to about 250-fold, from about 30-fold to about 250-fold, from about 35-fold to about 250-fold, from about 40-fold to about 250-fold, from about 50-fold to about 250-fold, from about 60-fold to about 250-fold, from about 70-fold to about 250-fold, from about 80-fold to about 250-fold, from about 90-fold to about 250-fold, from about 100-fold to about 250-fold, from about 150-fold to about 250-fold, or from about 200-fold to about 250-fold.

The amplification bias can be, for example, from about 1-fold to about 200-fold, from about 2-fold to about 200-fold, from about 3-fold to about 200-fold, from about 4-fold to about 200-fold, from about 5-fold to about 200-fold, from about 6-fold to about 200-fold, from about 7-fold to about 200-fold, from about 8-fold to about 200-fold, from about 9-fold to about 200-fold, from about 10-fold to about 200-fold, from about 11-fold to about 200-fold, from about 12-fold to about 200-fold, from about 14-fold to about 200-fold, from about 16-fold to about 200-fold, from about 20-fold to about 200-fold, from about 24-fold to about 200-fold, from about 30-fold to about 200-fold, from about 35-fold to about 200-

fold, from about 40-fold to about 200-fold, from about 50-fold to about 200-fold, from about 60-fold to about 200-fold, from about 70-fold to about 200-fold, from about 80-fold to about 200-fold, from about 90-fold to about 200-fold, from about 100-fold to about 200-fold, or from about 150-fold to about 200-fold.

The amplification bias can be, for example, from about 1-fold to about 150-fold, from about 2-fold to about 150-fold, from about 3-fold to about 150-fold, from about 4-fold to about 150-fold, from about 5-fold to about 150-fold, from about 6-fold to about 150-fold, from about 7-fold to about 150-fold, from about 8-fold to about 150-fold, from about 9-fold to about 150-fold, from about 10-fold to about 150-fold, from about 11-fold to about 150-fold, from about 12-fold to about 150-fold, from about 14-fold to about 150-fold, from about 16-fold to about 150-fold, from about 20-fold to about 150-fold, from about 24-fold to about 150-fold, from about 30-fold to about 150-fold, from about 35-fold to about 150-fold, from about 40-fold to about 150-fold, from about 50-fold to about 150-fold, from about 60-fold to about 150-fold, from about 70-fold to about 150-fold, from about 80-fold to about 150-fold, from about 90-fold to about 150-fold, or from about 100-fold to about 150-fold.

The amplification bias can be, for example, from about 1-fold to about 100-fold, from about 2-fold to about 100-fold, from about 3-fold to about 100-fold, from about 4-fold to about 100-fold, from about 5-fold to about 100-fold, from about 6-fold to about 100-fold, from about 7-fold to about 100-fold, from about 8-fold to about 100-fold, from about 9-fold to about 100-fold, from about 10-fold to about 100-fold, from about 11-fold to about 100-fold, from about 12-fold to about 100-fold, from about 14-fold to about 100-fold, from about 16-fold to about 100-fold, from about 20-fold to about 100-fold, from about 24-fold to about 100-fold, from about 30-fold to about 100-fold, from about 35-fold to about 100-fold, from about 40-fold to about 100-fold, from about 50-fold to about 100-fold, from about 60-fold to about 100-fold, from about 70-fold to about 100-fold, from about 80-fold to about 100-fold, or from about 90-fold to about 100-fold.

The amplification bias can be, for example, from about 1-fold to about 90-fold, from about 2-fold to about 90-fold, from about 3-fold to about 90-fold, from about 4-fold to about 90-fold, from about 5-fold to about 90-fold, from about 6-fold to about 90-fold, from about 7-fold to about 90-fold, from about 8-fold to about 90-fold, from about 9-fold to about 90-fold, from about 10-fold to about 90-fold, from about 11-fold to about 90-fold, from about 12-fold to about 90-fold, from about 14-fold to about 90-fold, from about 16-fold to about

90-fold, from about 20-fold to about 90-fold, from about 24-fold to about 90-fold, from about 30-fold to about 90-fold, from about 35-fold to about 90-fold, from about 40-fold to about 90-fold, from about 50-fold to about 90-fold, from about 60-fold to about 90-fold, from about 70-fold to about 90-fold, or from about 80-fold to about 90-fold.

The amplification bias can be, for example, from about 1-fold to about 80-fold, from about 2-fold to about 80-fold, from about 3-fold to about 80-fold, from about 4-fold to about 80-fold, from about 5-fold to about 80-fold, from about 6-fold to about 80-fold, from about 7-fold to about 80-fold, from about 8-fold to about 80-fold, from about 9-fold to about 80-fold, from about 10-fold to about 80-fold, from about 11-fold to about 80-fold, from about 12-fold to about 80-fold, from about 14-fold to about 80-fold, from about 16-fold to about 80-fold, from about 20-fold to about 80-fold, from about 24-fold to about 80-fold, from about 30-fold to about 80-fold, from about 35-fold to about 80-fold, from about 40-fold to about 80-fold, from about 50-fold to about 80-fold, from about 60-fold to about 80-fold, or from about 70-fold to about 80-fold.

The amplification bias can be, for example, from about 1-fold to about 70-fold, from about 2-fold to about 70-fold, from about 3-fold to about 70-fold, from about 4-fold to about 70-fold, from about 5-fold to about 70-fold, from about 6-fold to about 70-fold, from about 7-fold to about 70-fold, from about 8-fold to about 70-fold, from about 9-fold to about 70-fold, from about 10-fold to about 70-fold, from about 11-fold to about 70-fold, from about 12-fold to about 70-fold, from about 14-fold to about 70-fold, from about 16-fold to about 70-fold, from about 20-fold to about 70-fold, from about 24-fold to about 70-fold, from about 30-fold to about 70-fold, from about 35-fold to about 70-fold, from about 40-fold to about 70-fold, from about 50-fold to about 70-fold, or from about 60-fold to about 70-fold. The amplification bias can be, for example, from about 1-fold to about 60-fold, from about 2-fold to about 60-fold, from about 3-fold to about 60-fold, from about 4-fold to about 60-fold, from about 5-fold to about 60-fold, from about 6-fold to about 60-fold, from about 7-fold to about 60-fold, from about 8-fold to about 60-fold, from about 9-fold to about 60-fold, from about 10-fold to about 60-fold, from about 11-fold to about 60-fold, from about 12-fold to about 60-fold, from about 14-fold to about 60-fold, from about 16-fold to about 60-fold, from about 20-fold to about 60-fold, from about 24-fold to about 60-fold, from about 30-fold to about 60-fold, from about 35-fold to about 60-fold, from about 40-fold to about 60-fold, or from about 50-fold to about 60-fold.

The amplification bias can be, for example, from about 1-fold to about 50-fold, from about 2-fold to about 50-fold, from about 3-fold to about 50-fold, from about 4-fold to about 50-fold, from about 5-fold to about 50-fold, from about 6-fold to about 50-fold, from about 7-fold to about 50-fold, from about 8-fold to about 50-fold, from about 9-fold to about 50-

5      fold, from about 10-fold to about 50-fold, from about 11-fold to about 50-fold, from about 12-fold to about 50-fold, from about 14-fold to about 50-fold, from about 16-fold to about 50-fold, from about 20-fold to about 50-fold, from about 24-fold to about 50-fold, from about 30-fold to about 50-fold, from about 35-fold to about 50-fold, or from about 40-fold to about 50-fold. The amplification bias can be, for example, from about 1-fold to about 40-

10     fold, from about 2-fold to about 40-fold, from about 3-fold to about 40-fold, from about 4-fold to about 40-fold, from about 5-fold to about 40-fold, from about 6-fold to about 40-fold, from about 7-fold to about 40-fold, from about 8-fold to about 40-fold, from about 9-fold to about 40-fold, from about 10-fold to about 40-fold, from about 11-fold to about 40-fold, from about 12-fold to about 40-fold, from about 14-fold to about 40-fold, from about

15     16-fold to about 40-fold, from about 20-fold to about 40-fold, from about 24-fold to about 40-fold, from about 30-fold to about 40-fold, or from about 35-fold to about 40-fold.

The amplification bias can be, for example, from about 1-fold to about 30-fold, from about 2-fold to about 30-fold, from about 3-fold to about 30-fold, from about 4-fold to about 30-fold, from about 5-fold to about 30-fold, from about 6-fold to about 30-fold, from about

20     7-fold to about 30-fold, from about 8-fold to about 30-fold, from about 9-fold to about 30-fold, from about 10-fold to about 30-fold, from about 11-fold to about 30-fold, from about 12-fold to about 30-fold, from about 14-fold to about 30-fold, from about 16-fold to about 30-fold, from about 20-fold to about 30-fold, or from about 24-fold to about 30-fold. The amplification bias can be, for example, from about 1-fold to about 20-fold, from about 2-

25     fold to about 20-fold, from about 3-fold to about 20-fold, from about 4-fold to about 20-fold, from about 5-fold to about 20-fold, from about 6-fold to about 20-fold, from about 7-fold to about 20-fold, from about 8-fold to about 20-fold, from about 9-fold to about 20-fold, from about 10-fold to about 20-fold, from about 11-fold to about 20-fold, from about 12-fold to about 20-fold, from about 14-fold to about 20-fold, from about 16-fold to about

30     20-fold, from about 20-fold to about 20-fold, or from about 24-fold to about 20-fold.

The amplification bias can be, for example, from about 1-fold to about 12-fold, from about 2-fold to about 12-fold, from about 3-fold to about 12-fold, from about 4-fold to about 12-fold, from about 5-fold to about 12-fold, from about 6-fold to about 12-fold, from about

7-fold to about 12-fold, from about 8-fold to about 12-fold, from about 9-fold to about 12-fold, from about 10-fold to about 12-fold, or from about 11-fold to about 12-fold. The amplification bias can be, for example, from about 1-fold to about 11-fold, from about 2-fold to about 11-fold, from about 3-fold to about 11-fold, from about 4-fold to about 11-

5      fold, from about 5-fold to about 11-fold, from about 6-fold to about 11-fold, from about 7-fold to about 11-fold, from about 8-fold to about 11-fold, from about 9-fold to about 11-fold, or from about 10-fold to about 11-fold. The amplification bias can be, for example, from about 1-fold to about 10-fold, from about 2-fold to about 10-fold, from about 3-fold to about 10-fold, from about 4-fold to about 10-fold, from about 5-fold to about 10-fold, from

10     about 6-fold to about 10-fold, from about 7-fold to about 10-fold, from about 8-fold to about 10-fold, or from about 9-fold to about 10-fold. The amplification bias can be, for example, from about 1-fold to about 9-fold, from about 2-fold to about 9-fold, from about 3-fold to about 9-fold, from about 4-fold to about 9-fold, from about 5-fold to about 9-fold, from about 6-fold to about 9-fold, from about 7-fold to about 9-fold, or from about 8-fold to

15     about 9-fold.

The amplification bias can be, for example, from about 1-fold to about 8-fold, from about 2-fold to about 8-fold, from about 3-fold to about 8-fold, from about 4-fold to about 8-fold, from about 5-fold to about 8-fold, from about 6-fold to about 8-fold, or from about 7-fold to about 8-fold. The amplification bias can be, for example, from about 1-fold to

20     about 7-fold, from about 2-fold to about 7-fold, from about 3-fold to about 7-fold, from about 4-fold to about 7-fold, from about 5-fold to about 7-fold, or from about 6-fold to about 7-fold. The amplification bias can be, for example, from about 1-fold to about 6-fold, from about 2-fold to about 6-fold, from about 3-fold to about 6-fold, from about 4-fold to about 6-fold, or from about 5-fold to about 6-fold. The amplification bias can be, for

25     example, from about 1-fold to about 5-fold, from about 2-fold to about 5-fold, from about 3-fold to about 5-fold, from about 4-fold to about 5-fold, from about 1-fold to about 4-fold, from about 2-fold to about 4-fold, from about 3-fold to about 4-fold, from about 1-fold to about 3-fold, from about 2-fold to about 3-fold, or from about 1-fold to about 2-fold.

The various amplification biases described above and elsewhere herein can be, for

30     example, for 1 locus, 2 loci, 3 loci, 4 loci, 5 loci, 6 loci, 7 loci, 8 loci, 9 loci, 10 loci, 11 loci, 12 loci, 13 loci, 14 loci, 15 loci, 16 loci, 17 loci, 18 loci, 19 loci, 20 loci, 25 loci, 30 loci, 40 loci, 50 loci, 75 loci, or 100 loci. The amplification bias can be, for example, for at least 1 locus, at least 2 loci, at least 3 loci, at least 4 loci, at least 5 loci, at least 6 loci, at least 7

loci, at least 8 loci, at least 9 loci, at least 10 loci, at least 11 loci, at least 12 loci, at least 13 loci, at least 14 loci, at least 15 loci, at least 16 loci, at least 17 loci, at least 18 loci, at least 19 loci, at least 20 loci, at least 25 loci, at least 30 loci, at least 40 loci, at least 50 loci, at least 75 loci, or at least 100 loci.

5          The amplification bias can be, for example, for 1 locus, 2 different loci, 3 different loci, 4 different loci, 5 different loci, 6 different loci, 7 different loci, 8 different loci, 9 different loci, 10 different loci, 11 different loci, 12 different loci, 13 different loci, 14 different loci, 15 different loci, 16 different loci, 17 different loci, 18 different loci, 19 different loci, 20 different loci, 25 different loci, 30 different loci, 40 different loci, 50

10     different loci, 75 different loci, or 100 different loci. The amplification bias can be, for example, for at least 1 locus, at least 2 different loci, at least 3 different loci, at least 4 different loci, at least 5 different loci, at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different

15     loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

          The various amplification biases described above and elsewhere herein can be, for

20     example, for 1 target sequence, 2 target sequences, 3 target sequences, 4 target sequences, 5 target sequences, 6 target sequences, 7 target sequences, 8 target sequences, 9 target sequences, 10 target sequences, 11 target sequences, 12 target sequences, 13 target sequences, 14 target sequences, 15 target sequences, 16 target sequences, 17 target sequences, 18 target sequences, 19 target sequences, 20 target sequences, 25 target

25     sequences, 30 target sequences, 40 target sequences, 50 target sequences, 75 target sequences, or 100 target sequences. The amplification bias can be, for example, for at least 1 target sequence, at least 2 target sequences, at least 3 target sequences, at least 4 target sequences, at least 5 target sequences, at least 6 target sequences, at least 7 target sequences, at least 8 target sequences, at least 9 target sequences, at least 10 target sequences, at least

30     11 target sequences, at least 12 target sequences, at least 13 target sequences, at least 14 target sequences, at least 15 target sequences, at least 16 target sequences, at least 17 target sequences, at least 18 target sequences, at least 19 target sequences, at least 20 target sequences, at least 25 target sequences, at least 30 target sequences, at least 40 target

sequences, at least 50 target sequences, at least 75 target sequences, or at least 100 target sequences.

The amplification bias can be, for example, for 1 target sequence, 2 different target sequences, 3 different target sequences, 4 different target sequences, 5 different target sequences, 6 different target sequences, 7 different target sequences, 8 different target sequences, 9 different target sequences, 10 different target sequences, 11 different target sequences, 12 different target sequences, 13 different target sequences, 14 different target sequences, 15 different target sequences, 16 different target sequences, 17 different target sequences, 18 different target sequences, 19 different target sequences, 20 different target sequences, 25 different target sequences, 30 different target sequences, 40 different target sequences, 50 different target sequences, 75 different target sequences, or 100 different target sequences. The amplification bias can be, for example, for at least 1 target sequence, at least 2 different target sequences, at least 3 different target sequences, at least 4 different target sequences, at least 5 different target sequences, at least 6 different target sequences, at least 7 different target sequences, at least 8 different target sequences, at least 9 different target sequences, at least 10 different target sequences, at least 11 different target sequences, at least 12 different target sequences, at least 13 different target sequences, at least 14 different target sequences, at least 15 different target sequences, at least 16 different target sequences, at least 17 different target sequences, at least 18 different target sequences, at least 19 different target sequences, at least 20 different target sequences, at least 25 different target sequences, at least 30 different target sequences, at least 40 different target sequences, at least 50 different target sequences, at least 75 different target sequences, or at least 100 different target sequences.

## I. Nucleic Acid Sample Preparation and Treatment

Nucleic acids for amplification are often obtained from cellular samples. This generally requires disruption of the cell (to make the nucleic acid accessible) and purification of the nucleic acids prior to amplification. It also generally requires the inactivation of protein factors such as nucleases that could degrade the DNA, or of factors such as histones that could bind to DNA strands and impede their use as a template for DNA synthesis by a polymerase. There are a variety of techniques used to break open cells, such as sonication, enzymatic digestion of cell walls, heating, and exposure to lytic conditions. Lytic conditions typically involve use of non-physiological pH and/or solvents. Many lytic techniques can result in damage to nucleic acids in cells, including, for example,

breakage of genomic DNA. In particular, use of heating to lyse cells can damage genomic DNA and reduce the amount and quality of amplification products of genomic DNA. It has been discovered that alkaline lysis can cause less damage to genomic DNA and can thus result in higher quality amplification results. Alkaline lysis also inactivates protein factors

5    such as nucleases, histones, or other factors that could impede the amplification of DNA within the sample. In addition, it is a useful property of alkaline lysis that reducing the pH does not reactivate the protein factors, but that such protein factors remain inactivated when the pH of the solution is brought back within a neutral range.

In some forms of the disclosed method, a genomic sample is prepared by exposing

10    cells to alkaline conditions, thereby lysing the cells and resulting in a cell lysate; reducing the pH of the cell lysate to make the pH of the cell lysate compatible with DNA replication; and incubating the cell lysate under conditions that promote replication of the genome of the cells by multiple displacement amplification. Alkaline conditions are conditions where the pH is greater than 9.0. Particularly useful alkaline conditions for the disclosed method are

15    conditions where the pH is greater than 10.0. The alkaline conditions can be, for example, those that cause a substantial number of cells to lyse, those that cause a significant number of cells to lyse, or those that cause a sufficient number of cells to lyse. The number of lysed cells can be considered sufficient if the genome can be sufficiently amplified in the disclosed method. The amplification is sufficient if enough amplification product is

20    produced to permit some use of the amplification product, such as detection of sequences or other analysis. The reduction in pH is generally into the neutral range of pH 9.0 to pH 6.0.

The cells can be exposed to alkaline conditions by mixing the cells with a lysis solution. The amount of lysis solution mixed with the cells can be that amount that causes a substantial number of cells to lyse or those that cause a sufficient number of cells to lyse.

25    Generally, this volume will be a function of the pH of the cell/lysis solution mixture. Thus, the amount of lysis solution to mix with cells can be determined generally from the volume of the cells and the alkaline concentration of the lysis buffer. For example, a smaller volume of a lysis solution with a stronger base and/or higher concentration of base would be needed to create sufficient alkaline conditions than the volume needed of a lysis solution

30    with a weaker base and/or lower concentration of base. The lysis solution can be formulated such that the cells are mixed with an equal volume of the lysis solution (to produce the desired alkaline conditions).

In some embodiments, the lysis solution can comprise a base, such as an aqueous base. Useful bases include potassium hydroxide, sodium hydroxide, potassium acetate, sodium acetate, ammonium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, calcium carbonate, ammonia, aniline,

5      benzylamine, n-butylamine, diethylamine, dimethylamine, diphenylamine, ethylamine, ethylenediamine, methylamine, N-methylaniline, morpholine, pyridine, triethylamine, trimethylamine, aluminum hydroxide, rubidium hydroxide, cesium hydroxide, strontium hydroxide, barium hydroxide, and DBU (1,8-diazobicyclo[5,4,0]undec-7-ene). Useful formulations of lysis solution include lysis solution comprising 400 mM KOH, lysis

10     solution comprising 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA, and lysis solution consisting of 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA.

In some embodiments, the lysis solution can comprise a plurality of basic agents. As used herein, a basic agent is a compound, composition or solution that results in alkaline conditions. In some embodiments, the lysis solution can comprise a buffer. Useful buffers

15     include phosphate buffers, "Good" buffers (such as BES, BICINE, CAPS, EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES, and TRICINE), sodium cacodylate, sodium citrate, triethylammonium acetate, triethylammonium bicarbonate, Tris, Bis-tris, and Bis-tris propane. The lysis solution can comprise a plurality of buffering agents. As used herein, a buffering agent is a compound, composition or solution that acts as a buffer. An alkaline

20     buffering agent is a buffering agent that results in alkaline conditions. In some embodiments, the lysis solution can comprise a combination of one or more bases, basic agents, buffers and buffering agents.

The pH of the cell lysate can be reduced to form a stabilized cell lysate. A stabilized cell lysate is a cell lysate the pH of which is in the neutral range (from about pH 6.0 to about

25     pH 9.0). Useful stabilized cell lysates have a pH that allows replication of nucleic acids in the cell lysate. For example, the pH of the stabilized cell lysate is usefully at a pH at which the DNA polymerase can function. The pH of the cell lysate can be reduced by mixing the cell lysate with a stabilization solution. The stabilization solution comprises a solution that can reduce the pH of a cell lysate exposed to alkaline conditions as described elsewhere

30     herein.

The amount of stabilization solution mixed with the cell lysate can be that amount that causes a reduction in pH to the neutral range (or other desired pH value). Generally, this volume will be a function of the pH of the cell lysate/stabilization solution mixture.

Thus, the amount of stabilization solution to mix with the cell lysate can be determined generally from the volume of the cell lysate, its pH and buffering capacity, and the acidic concentration of the stabilization buffer. For example, a smaller volume of a stabilization solution with a stronger acid and/or higher concentration of acid would be needed to reduce

5      the pH sufficiently than the volume needed of a stabilization solution with a weaker acid and/or lower concentration of acid. The stabilization solution can be formulated such that the cell lysate is mixed with an equal volume of the stabilization solution (to produce the desired pH).

In some embodiments, the stabilization solution can comprise an acid. Useful acids

10     include hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid, acetylsalicylic acid, ascorbic acid, carbonic acid, citric acid, formic acid, nitric acid, perchloric acid, HF, HBr, HI, $H_2S$, HCN, HSCN, HClO, monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, and any carboxylic acid (ethanoic, propanoic, butanoic, etc., including both linear or branched chain carboxylic acids). In some embodiments, the stabilization solution can

15     comprise a buffer. Useful buffers include Tris-HCl, HEPES, "Good" buffers (such as BES, BICINE, CAPS, EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES, and TRICINE), sodium cacodylate, sodium citrate, triethylammonium acetate, triethylammonium bicarbonate, Tris, Bis-tris, and Bis-tris propane. Useful formulations of stabilization solutions include stabilization solution comprising 800 mM Tris-HCl; stabilization solution comprising 800

20     mM Tris-HCl at pH 4.1; and stabilization solution consisting of 800 mM Tris-HCl, pH 4.1.

In some embodiments, the stabilization solution can comprise a plurality of acidic agents. As used herein, an acidic agent is a compound, composition or solution that forms an acid in solution. In some embodiments, the stabilization solution can comprise a plurality of buffering agents. An acidic buffering agent is a buffering agent that forms an

25     acid in solution. In some embodiments, the stabilization solution can comprise a combination of one or more acids, acidic agents, buffers and buffering agents.

In some embodiments, the pH of the cell lysate can be reduced to about pH 9.0 or below, to about pH 8.5 or below, to about pH 8.0 or below, to about pH 7.5 or below, to about pH 7.2 or below, or to about pH 7.0 or below. In some embodiments, the pH of the

30     cell lysate can be reduced to the range of about pH 9.0 to about pH 6.0, to the range of about pH 9.0 to about pH 6.5, to the range of about pH 9.0 to about pH 6.8, to the range of about pH 9.0 to about pH 7.0, to the range of about pH 9.0 to about pH 7.2, to the range of about pH 9.0 to about pH 7.5, to the range of about pH 9.0 to about pH 8.0, to the range of about

pH 9.0 to about pH 8.5, to the range of about pH 8.5 to about pH 6.0, to the range of about

pH 8.5 to about pH 6.5, to the range of about pH 8.5 to about pH 6.8, to the range of about

pH 8.5 to about pH 7.0, to the range of about pH 8.5 to about pH 7.2, to the range of about

pH 8.5 to about pH 7.5, to the range of about pH 8.5 to about pH 8.0, to the range of about

5      pH 8.0 to about pH 6.0, to the range of about pH 8.0 to about pH 6.5, to the range of about

pH 8.0 to about pH 6.8, to the range of about pH 8.0 to about pH 7.0, to the range of about

pH 8.0 to about pH 7.2, to the range of about pH 8.0 to about pH 7.5, to the range of about

pH 7.5 to about pH 6.0, to the range of about pH 7.5 to about pH 6.5, to the range of about

pH 7.5 to about pH 6.8, to the range of about pH 7.5 to about pH 7.0, to the range of about

10     pH 7.5 to about pH 7.2, to the range of about pH 7.2 to about pH 6.0, to the range of about

pH 7.2 to about pH 6.5, to the range of about pH 7.2 to about pH 6.8, to the range of about

pH 7.2 to about pH 7.0, to the range of about pH 7.0 to about pH 6.0, to the range of about

pH 7.0 to about pH 6.5, to the range of about pH 7.0 to about pH 6.8, to the range of about

pH 6.8 to about pH 6.0, to the range of about pH 6.8 to about pH 6.5, or to the range of

15     about pH 6.5 to about pH 6.0.  In some embodiments, the pH of the cell lysate can be

reduced to any range having any combination of endpoints from about pH 6.0 to about pH

9.0  All such endpoints and ranges are specifically and separately contemplated.

In some embodiments, the cells are not lysed by heat.  Those of skill in the art will

understand that different cells under different conditions will be lysed at different

20     temperatures and so can determine temperatures and times at which the cells will not be

lysed by heat.  In general, the cells are not subjected to heating above a temperature and for

a time that would cause substantial cell lysis in the absence of the alkaline conditions used.

As used herein, substantial cell lysis refers to lysis of 90% or greater of the cells exposed to

the alkaline conditions.  Significant cell lysis refers to lysis of 50% or more of the cells

25     exposed to the alkaline conditions.  Sufficient cell lysis refers to lysis of enough of the cells

exposed to the alkaline conditions to allow synthesis of a detectable amount of amplification

products by multiple strand displacement amplification.  In general, the alkaline conditions

used in the disclosed method need only cause sufficient cell lysis.  It should be understood

that alkaline conditions that could cause significant or substantial cell lysis need not result

30     in significant or substantial cell lysis when the method is performed.

In some embodiments, the cells are not subjected to heating substantially or

significantly above the temperature at which the cells grow.  As used herein, the

temperature at which the cells grow refers to the standard temperature, or highest of

different standard temperatures, at which cells of the type involved are cultured. In the case of animal cells, the temperature at which the cells grow refers to the body temperature of the animal. In other embodiments, the cells are not subjected to heating substantially or significantly above the temperature of the amplification reaction (where the genome is

5    replicated).

In some embodiments, the cell lysate is not subjected to purification prior to the amplification reaction. In the context of the disclosed method, purification generally refers to the separation of nucleic acids from other material in the cell lysate. It has been discovered that multiple displacement amplification can be performed on unpurified and

10   partially purified samples. It is commonly thought that amplification reactions cannot be efficiently performed using unpurified nucleic acid. In particular, PCR is very sensitive to contaminants.

Forms of purification include centrifugation, extraction, chromatography, precipitation, filtration, and dialysis. Partially purified cell lysate includes cell lysates

15   subjected to centrifugation, extraction, chromatography, precipitation, filtration, and dialysis. Partially purified cell lysate generally does not include cell lysates subjected to nucleic acid precipitation or dialysis. As used herein, separation of nucleic acid from other material refers to physical separation such that the nucleic acid to be amplified is in a different container or container from the material. Purification does not require separation

20   of all nucleic acid from all other materials. Rather, what is required is separation of some nucleic acid from some other material. As used herein in the context of nucleic acids to be amplified, purification refers to separation of nucleic acid from other material. In the context of cell lysates, purification refers to separation of nucleic acid from other material in the cell lysate. As used herein, partial purification refers to separation of nucleic acid from

25   some, but not all, of other material with which the nucleic acid is mixed. In the context of cell lysates, partial purification refers to separation of nucleic acid from some, but not all, of the other material in the cell lysate.

Unless the context clearly indicates otherwise, reference herein to a lack of purification, lack of one or more types of purification or separation operations or

30   techniques, or exclusion of purification or one or more types of purification or separation operations or techniques does not encompass the exposure of cells to alkaline conditions (or the results thereof) the reduction of pH of a cell lysate (or the results thereof). That is, to the extent that the alkaline conditions and pH reduction of the disclosed method produce an

185

effect that could be considered "purification" or "separation," such effects are excluded from the definition of purification and separation when those terms are used in the context of processing and manipulation of cell lysates and stabilized cell lysates (unless the context clearly indicates otherwise).

5          As used herein, substantial purification refers to separation of nucleic acid from at least a substantial portion of other material with which the nucleic acid is mixed. In the context of cell lysates, substantial purification refers to separation of nucleic acid from at least a substantial portion of the other material in the cell lysate. A substantial portion refers to 90% of the other material involved. Specific levels of purification can be referred to as a

10        percent purification (such as 95% purification and 70% purification). A percent purification refers to purification that results in separation from nucleic acid of at least the designated percent of other material with which the nucleic acid is mixed.

Denaturation of nucleic acid molecules to be amplified is common in amplification techniques. This is especially true when amplifying genomic DNA. In particular, PCR uses

15        multiple denaturation cycles. Denaturation is generally used to make nucleic acid strands accessible to primers. Nucleic acid molecules, genomic DNA, for example, need not be denatured for efficient multiple displacement amplification. Elimination of a denaturation step and denaturation conditions has additional advantages such as reducing sequence bias in the amplified products (that is, reducing the amplification bias). In preferred forms of the

20        disclosed method, the nucleic acid sample or template nucleic acid is not subjected to denaturating conditions and/or no denaturation step is used.

In some forms of the disclosed method, the nucleic acid sample or template nucleic acid is not subjected to heat denaturating conditions and/or no heat denaturation step is used. In some forms of the disclosed method, the nucleic acid sample or template nucleic

25        acid is not subjected to alkaline denaturating conditions and/or no alkaline denaturation step is used. It should be understood that while sample preparation (for example, cell lysis and processing of cell extracts) may involve conditions that might be considered denaturing (for example, treatment with alkali), the denaturation conditions or step eliminated in some forms of the disclosed method refers to denaturation steps or conditions intended and used

30        to make nucleic acid strands accessible to primers. Such denaturation is commonly a heat denaturation, but can also be other forms of denaturation such as chemical denaturation. It should be understood that in the disclosed method where the nucleic acid sample or template nucleic acid is not subjected to denaturing conditions, the template strands are

accessible to the primers (since amplification occurs). However, the template stands are not made accessible via general denaturation of the sample or template nucleic acids.

Alternatively, the nucleic acid sample or template nucleic acid can be subjected to denaturing conditions and/or a denaturation step can be used. In some forms of the

5      disclosed method, the nucleic acid sample or template nucleic acid can be subjected to heat denaturating conditions and/or a heat denaturation step can be used. In some forms of the disclosed method, the nucleic acid sample or template nucleic acid can be subjected to alkaline denaturating conditions and/or an alkaline denaturation step can be used.

The efficiency of a DNA amplification procedure may be described for individual

10     loci as the percent representation of that locus (that is, the locus representation), where the locus representation is 100% for a locus in genomic DNA as purified from cells. For 10,000-fold amplification, the average representation frequency was 141% for 8 loci in DNA amplified without heat denaturation of the template, and 37% for the 8 loci in DNA amplified with heat denaturation of the template. The omission of a heat denaturation step

15     results in a 3.8-fold increase in the representation frequency for amplified loci. Amplification bias may be calculated between two samples of amplified DNA or between a sample of amplified DNA and the template DNA it was amplified from. The bias is the ratio between the values for percent representation for a particular locus (locus representation). The maximum bias is the ratio of the most highly represented locus to the least represented

20     locus. For 10,000-fold amplification, the maximum amplification bias was 2.8 for DNA amplified without heat denaturation of the template, and 50.7 for DNA amplified with heat denaturation of the template. The omission of a heat denaturation step results in an 18-fold decrease in the maximum bias for amplified loci.

In one form of the disclosed method, a small amount of purified double-strand

25     human genomic DNA (1 ng, for example) can be mixed with one or a few exonuclease-resistant primers 6 nucleotides long and φ29 DNA polymerase under conditions that favor DNA synthesis. For example, the mixture can simply be incubated at 30°C and multiple displacement amplification will take place. Thus, any single-stranded or duplex DNA may be used, without any additional treatment, making the disclosed method a simple, one-step

30     procedure. Since so little DNA template is required, a major advantage of the disclosed method is that DNA template may be taken from preparations that contain levels of contaminants that would inhibit other DNA amplification procedures such as PCR. For MDA the sample may be diluted so that the contaminants fall below the concentration at

which they would interfere with the reaction. The disclosed method can be performed (and the above advantages achieved) using any type of sample, including, for example, bodily fluids such as urine, semen, lymphatic fluid, cerebrospinal fluid, and amniotic fluid.

The need for only small amounts of DNA template in the disclosed method means
5        that the method is useful for DNA amplification from very small samples. In particular, the disclosed method may be used to amplify DNA from a single cell. The ability to obtain analyzable amounts of nucleic acid from a single cell (or similarly small sample) has many applications in preparative, analytical, and diagnostic procedures such as prenatal diagnostics. Other examples of biological samples containing only small amounts of DNA
10       for which amplification by the disclosed method would be useful are material excised from tumors or other archived medical samples, needle aspiration biopsies, clinical samples arising from infections, such as nosocomial infections, forensic samples, or museum specimens of extinct species.

More broadly, the disclosed method is useful for applications in which the amounts
15       of DNA needed are greater than the supply. For example, procedures that analyze DNA by chip hybridization techniques are limited by the amounts of DNA that can be purified from typically sized blood samples. As a result many chip hybridization procedures utilize PCR to generate a sufficient supply of material for the high-throughput procedures. The disclosed method presents a useful technique for the generation of plentiful amounts of
20       amplified DNA that faithfully reproduces the locus representation frequencies of the starting material.

The disclosed method can produce a DNA amplification product with improved performance in genetic assays compared to amplification performed with heat treatment of the template DNA. The longer DNA products produced without heat treatment of the
25       template yield larger DNA fragments in Southern blotting and genetic analysis using RFLP. The disclosed method produces for a DNA amplification product with no loss of locus representation when used as a substrate in quantitative PCR assays compared to DNA amplified with heat treatment of the template. The disclosed method produces a DNA amplification product with a low amplification bias, with the variation in representation
30       among eight different loci varying by less than 3.0. In contrast, the amplification bias of DNA products amplified by two PCR-based amplification methods, PEP and DOP-PCR, varies between two and six orders of magnitude.

Another specific form of the disclosed method involves amplification of genomic DNA the absence of a heat treatment step directly from whole blood or from tissue culture cells. Such amplification can be achieved with the same efficiency as from purified DNA. The DNA amplified directly from blood or cells can have substantially the same locus

5      representation values as DNA amplified from purified human DNA template. This represents an advantage over other amplification procedures such as PCR, since components such as heme in whole blood inhibit PCR and necessitate a purification step before DNA from blood can be used as a PCR template.

**J.    Detection of Amplification Products**

10     Products of amplification can be detected using any nucleic acid detection technique. For real-time detection, the amplification products and the progress of amplification are detected during amplification. Real-time detection is usefully accomplished using one or more or one or a combination of fluorescent change probes and fluorescent change primers. Other detection techniques can be used, either alone or in

15     combination with real-timer detection and/or detection involving fluorescent change probes and primers. Many techniques are known for detecting nucleic acids. The nucleotide sequence of the amplified sequences also can be determined using any suitable technique.

**K.    Modifications And Additional Operations**

   **1.    Detection of Amplification Products**

20     Amplification products can be detected directly by, for example, primary labeling or secondary labeling, as described below.

   **i.    Primary Labeling**

Primary labeling consists of incorporating labeled moieties, such as fluorescent nucleotides, biotinylated nucleotides, digoxygenin-containing nucleotides, or

25     bromodeoxyuridine, during strand displacement replication. For example, one may incorporate cyanine dye deoxyuridine analogs (Yu *et al.*, *Nucleic Acids Res.*, **22**:3226-3232 (1994)) at a frequency of 4 analogs for every 100 nucleotides. A preferred method for detecting nucleic acid amplified *in situ* is to label the DNA during amplification with BrdUrd, followed by binding of the incorporated BrdU with a biotinylated anti-BrdU

30     antibody (Zymed Labs, San Francisco, CA), followed by binding of the biotin moieties with Streptavidin-Peroxidase (Life Sciences, Inc.), and finally development of fluorescence with Fluorescein-tyramide (DuPont de Nemours & Co., Medical Products Dept.). Other methods for detecting nucleic acid amplified *in situ* include labeling the DNA during amplification

with 5-methylcytosine, followed by binding of the incorporated 5-methylcytosine with an antibody (Sano *et al., Biochim. Biophys. Acta* **951**:157-165 (1988)), or labeling the DNA during amplification with aminoallyl-deoxyuridine, followed by binding of the incorporated aminoallyl-deoxyuridine with an Oregon Green® dye (Molecular Probes, Eugene, OR)

5       (Henegariu *et al., Nature Biotechnology* **18**:345-348 (2000)).

Another method of labeling amplified nucleic acids is to incorporate 5-(3-aminoallyl)-dUTP (AAdUTP) in the nucleic acid during amplification followed by chemical labeling at the incorporated nucleotides. Incorporated 5-(3-aminoallyl)-deoxyuridine (AAdU) can be coupled to labels that have reactive groups that are capable of reacting with

10      amine groups. AAdUTP can be prepared according to Langer et al. (1981). Proc. Natl. Acad. Sci. USA. 78: 6633-37. Other modified nucleotides can be used in analogous ways. That is, other modified nucleotides with minimal modification can be incorporated during replication and labeled after incorporation.

Examples of labels suitable for addition to AAdUTP are radioactive isotopes,

15      fluorescent molecules, phosphorescent molecules, enzymes, antibodies, and ligands. Examples of suitable fluorescent labels include fluorescein isothiocyanate (FITC), 5,6-carboxymethyl fluorescein, Texas red, nitrobenz-2-oxa-1,3-diazol-4-yl (NBD), coumarin, dansyl chloride, rhodamine, amino-methyl coumarin (AMCA), Eosin, Erythrosin, BODIPY®, Cascade Blue®, Oregon Green®, pyrene, lissamine, xanthenes, acridines,

20      oxazines, phycoerythrin, macrocyclic chelates of lanthanide ions such as quantum dye™, fluorescent energy transfer dyes, such as thiazole orange-ethidium heterodimer, and the cyanine dyes Cy3, Cy3.5, Cy5, Cy5.5 and Cy7. Examples of other specific fluorescent labels include 3-Hydroxypyrene 5,8,10-Tri Sulfonic acid, 5-Hydroxy Tryptamine (5-HT), Acid Fuchsin, Alizarin Complexon, Alizarin Red, Allophycocyanin, Aminocoumarin,

25      Anthroyl Stearate, Astrazon Brilliant Red 4G, Astrazon Orange R, Astrazon Red 6B, Astrazon Yellow 7 GLL, Atabrine, Auramine, Aurophosphine, Aurophosphine G, BAO 9 (Bisaminophenyloxadiazole), BCECF, Berberine Sulphate, Bisbenzamide, Blancophor FFG Solution, Blancophor SV, Bodipy F1, Brilliant Sulphoflavin FF, Calcien Blue, Calcium Green, Calcofluor RW Solution, Calcofluor White, Calcophor White ABT Solution,

30      Calcophor White Standard Solution, Carbostyryl, Cascade Yellow, Catecholamine, Chinacrine, Coriphosphine O, Coumarin-Phalloidin, CY3.1 8, CY5.1 8, CY7, Dans (1-Dimethyl Amino Naphaline 5 Sulphonic Acid), Dansa (Diamino Naphtyl Sulphonic Acid), Dansyl NH-CH3, Diamino Phenyl Oxydiazole (DAO), Dimethylamino-5-Sulphonic acid,

Dipyrrometheneboron Difluoride, Diphenyl Brilliant Flavine 7GFF, Dopamine, Erythrosin

ITC, Euchrysin, FIF (Formaldehyde Induced Fluorescence), Flazo Orange, Fluo 3,

Fluorescamine, Fura-2, Genacryl Brilliant Red B, Genacryl Brilliant Yellow 10GF,

Genacryl Pink 3G, Genacryl Yellow 5GF, Gloxalic Acid, Granular Blue,

5        Haematoporphyrin, Indo-1, Intrawhite Cf Liquid, Leucophor PAF, Leucophor SF,

Leucophor WS, Lissamine Rhodamine B200 (RD200), Lucifer Yellow CH, Lucifer Yellow

VS, Magdala Red, Marina Blue, Maxilon Brilliant Flavin 10 GFF, Maxilon Brilliant Flavin

8 GFF, MPS (Methyl Green Pyronine Stilbene), Mithramycin, NBD Amine,

Nitrobenzoxadidole, Noradrenaline, Nuclear Fast Red, Nuclear Yellow, Nylosan Brilliant

10       Flavin E8G, Oxadiazole, Pacific Blue, Pararosaniline (Feulgen), Phorwite AR Solution,

Phorwite BKL, Phorwite Rev, Phorwite RPA, Phosphine 3R, Phthalocyanine,

Phycoerythrin R, Polyazaindacene Pontochrome Blue Black, Porphyrin, Primuline, Procion

Yellow, Pyronine, Pyronine B, Pyrozal Brilliant Flavin 7GF, Quinacrine Mustard,

Rhodamine 123, Rhodamine 5 GLD, Rhodamine 6G, Rhodamine B, Rhodamine B 200,

15       Rhodamine B Extra, Rhodamine BB, Rhodamine BG, Rhodamine WT, Serotonin, Sevron

Brilliant Red 2B, Sevron Brilliant Red 4G, Sevron Brilliant Red B, Sevron Orange, Sevron

Yellow L, SITS (Primuline), SITS (Stilbene Isothiosulphonic acid), Stilbene, Snarf 1,

sulpho Rhodamine B Can C, Sulpho Rhodamine G Extra, Tetracycline, Thiazine Red R,

Thioflavin S, Thioflavin TCN, Thioflavin 5, Thiolyte, Thiozol Orange, Tinopol CBS, True

20       Blue, Ultralite, Uranine B, Uvitex SFC, Xylene Orange, and XRITC.

Preferred fluorescent labels are fluorescein (5-carboxyfluorescein-N-

hydroxysuccinimide ester), rhodamine (5,6-tetramethyl rhodamine), and the cyanine dyes

Cy3, Cy3.5, Cy5, Cy5.5 and Cy7.  The absorption and emission maxima, respectively, for

these fluors are: FITC (490 nm; 520 nm), Cy3 (554 nm; 568 nm), Cy3.5 (581 nm; 588 nm),

25       Cy5 (652 nm: 672 nm), Cy5.5 (682 nm; 703 nm) and Cy7 (755 nm; 778 nm), thus allowing

their simultaneous detection.  Other examples of fluorescein dyes include 6-

carboxyfluorescein (6-FAM), 2',4',1,4,-tetrachlorofluorescein (TET), 2',4',5',7',1,4-

hexachlorofluorescein (HEX), 2',7'-dimethoxy-4', 5'-dichloro-6-carboxyrhodamine (JOE),

2'-chloro-5'-fluoro-7',8'-fused phenyl-1,4-dichloro-6-carboxyfluorescein (NED), and 2'-

30       chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC).  Fluorescent labels can be

obtained from a variety of commercial sources, including Amersham Pharmacia Biotech,

Piscataway, NJ; Molecular Probes, Eugene, OR; and Research Organics, Cleveland, Ohio.

A useful form of primary labeling is the use of fluorescent change primers during amplification. Fluorescent change primers exhibit a change in fluorescence intensity or wavelength based on a change in the form or conformation of the primer and the amplified nucleic acid. Stem quenched primers are primers that when not hybridized to a

5      complementary sequence form a stem structure (either an intramolecular stem structure or an intermolecular stem structure) that brings a fluorescent label and a quenching moiety into proximity such that fluorescence from the label is quenched. When the primer binds to a complementary sequence, the stem is disrupted, the quenching moiety is no longer in proximity to the fluorescent label and fluorescence increases. In the disclosed method, stem

10     quenched primers can be used as primers for nucleic acid synthesis and thus become incorporated into the synthesized or amplified nucleic acid. Examples of stem quenched primers are peptide nucleic acid quenched primers and hairpin quenched primers.

       Peptide nucleic acid quenched primers are primers associated with a peptide nucleic acid quencher or a peptide nucleic acid fluor to form a stem structure. The primer contains

15     a fluorescent label or a quenching moiety and is associated with either a peptide nucleic acid quencher or a peptide nucleic acid fluor, respectively. This puts the fluorescent label in proximity to the quenching moiety. When the primer is replicated, the peptide nucleic acid is displaced, thus allowing the fluorescent label to produce a fluorescent signal.

       Hairpin quenched primers are primers that when not hybridized to a complementary

20     sequence form a hairpin structure (and, typically, a loop) that brings a fluorescent label and a quenching moiety into proximity such that fluorescence from the label is quenched. When the primer binds to a complementary sequence, the stem is disrupted, the quenching moiety is no longer in proximity to the fluorescent label and fluorescence increases. Hairpin quenched primers are typically used as primers for nucleic acid synthesis and thus become

25     incorporated into the synthesized or amplified nucleic acid. Examples of hairpin quenched primers are Amplifluor primers and scorpion primers.

       Cleavage activated primers are primers where fluorescence is increased by cleavage of the primer. Generally, cleavage activated primers are incorporated into replicated strands and are then subsequently cleaved. Cleavage activated primers can include a fluorescent

30     label and a quenching moiety in proximity such that fluorescence from the label is quenched. When the primer is clipped or digested (typically by the 5'-3' exonuclease activity of a polymerase during amplification), the quenching moiety is no longer in proximity to the fluorescent label and fluorescence increases. Little et al., Clin. Chem.

45:777-784 (1999), describe the use of cleavage activated primers. Use of cleavage activated primers is not preferred in the disclosed method.

### ii. Secondary Labeling with Detection Probes

Secondary labeling consists of using suitable molecular probes, referred to as detection probes, to detect the amplified nucleic acids. For example, a primer may be designed to contain, in its non-complementary portion, a known arbitrary sequence, referred to as a detection tag. A secondary hybridization step can be used to bind detection probes to these detection tags. The detection probes may be labeled as described above with, for example, an enzyme, fluorescent moieties, or radioactive isotopes. By using three detection tags per primer, and four fluorescent moieties per each detection probe, one may obtain a total of twelve fluorescent signals for every replicated strand. Detection probes can interact by hybridization or annealing via normal Watson-Crick base-pairing (or related alternatives) or can interact with double-stranded targets to form a triple helix. Such triplex-forming detection probes can be used in the same manner as other detection probes, such as in the form of fluorescent change probes.

A useful form of secondary labeling is the use of fluorescent change probes and primers in or following amplification. Hairpin quenched probes are probes that when not bound to a target sequence form a hairpin structure (and, typically, a loop) that brings a fluorescent label and a quenching moiety into proximity such that fluorescence from the label is quenched. When the probe binds to a target sequence, the stem is disrupted, the quenching moiety is no longer in proximity to the fluorescent label and fluorescence increases. Examples of hairpin quenched probes are molecular beacons, fluorescent triplex oligos, and QPNA probes.

Cleavage activated probes are probes where fluorescence is increased by cleavage of the probe. Cleavage activated probes can include a fluorescent label and a quenching moiety in proximity such that fluorescence from the label is quenched. When the probe is clipped or digested (typically by the 5'-3' exonuclease activity of a polymerase during or following amplification), the quenching moiety is no longer in proximity to the fluorescent label and fluorescence increases. TaqMan probes are an example of cleavage activated probes.

Cleavage quenched probes are probes where fluorescence is decreased or altered by cleavage of the probe. Cleavage quenched probes can include an acceptor fluorescent label and a donor moiety such that, when the acceptor and donor are in proximity, fluorescence

resonance energy transfer from the donor to the acceptor causes the acceptor to fluoresce. The probes are thus fluorescent, for example, when hybridized to a target sequence. When the probe is clipped or digested (typically by the 5'-3' exonuclease activity of a polymerase during or after amplification), the donor moiety is no longer in proximity to the acceptor

5       fluorescent label and fluorescence from the acceptor decreases. If the donor moiety is itself a fluorescent label, it can release energy as fluorescence (typically at a different wavelength than the fluorescence of the acceptor) when not in proximity to an acceptor. The overall effect would then be a reduction of acceptor fluorescence and an increase in donor fluorescence. Donor fluorescence in the case of cleavage quenched probes is equivalent to

10      fluorescence generated by cleavage activated probes with the acceptor being the quenching moiety and the donor being the fluorescent label. Cleavable FRET (fluorescence resonance energy transfer) probes are an example of cleavage quenched probes.

Fluorescent activated probes are probes or pairs of probes where fluorescence is increased or altered by hybridization of the probe to a target sequence. Fluorescent

15      activated probes can include an acceptor fluorescent label and a donor moiety such that, when the acceptor and donor are in proximity (when the probes are hybridized to a target sequence), fluorescence resonance energy transfer from the donor to the acceptor causes the acceptor to fluoresce. Fluorescent activated probes are typically pairs of probes designed to hybridize to adjacent sequences such that the acceptor and donor are brought into proximity.

20      Fluorescent activated probes can also be single probes containing both a donor and acceptor where, when the probe is not hybridized to a target sequence, the donor and acceptor are not in proximity but where the donor and acceptor are brought into proximity when the probe hybridized to a target sequence. This can be accomplished, for example, by placing the donor and acceptor on opposite ends a the probe and placing target complement sequences

25      at each end of the probe where the target complement sequences are complementary to adjacent sequences in a target sequence. If the donor moiety of a fluorescent activated probe is itself a fluorescent label, it can release energy as fluorescence (typically at a different wavelength than the fluorescence of the acceptor) when not in proximity to an acceptor (that is, when the probes are not hybridized to the target sequence). When the

30      probes hybridize to a target sequence, the overall effect would then be a reduction of donor fluorescence and an increase in acceptor fluorescence. FRET probes are an example of fluorescent activated probes. Stem quenched primers (such as peptide nucleic acid quenched primers and hairpin quenched primers) can be used as secondary labels.

194

### iii. Multiplexing and Hybridization Array Detection

Detection of amplified nucleic acids can be multiplexed by using sets of different primers, each set designed for amplifying different target sequences. Only those primers that are able to find their targets will give rise to amplified products. There are two

5    alternatives for capturing a given amplified nucleic acid to a fixed position in a solid-state detector. One is to include within the non-complementary portion of the primers a unique address tag sequence for each unique set of primers. Nucleic acid amplified using a given set of primers will then contain sequences corresponding to a specific address tag sequence. A second and preferred alternative is to use a sequence present in the target sequence as an

10   address tag. The disclosed method can be easily multiplexed by, for example, using sets of different detection probes directed to different target sequences. Use of different fluorescent labels with different detection probes allows specific detection of different target sequences.

### 2. Combinatorial Multicolor Coding

15        One form of multiplex detection involves the use of a combination of labels that either fluoresce at different wavelengths or are colored differently. One of the advantages of fluorescence for the detection of hybridization probes is that several targets can be visualized simultaneously in the same sample. Using a combinatorial strategy, many more targets can be discriminated than the number of spectrally resolvable fluorophores.

20   Combinatorial labeling provides the simplest way to label probes in a multiplex fashion since a probe fluor is either completely absent (-) or present in unit amounts (+); image analysis is thus more amenable to automation, and a number of experimental artifacts, such as differential photobleaching of the fluors and the effects of changing excitation source power spectrum, are avoided. Combinatorial labeling can be used with fluorescent change

25   probes and primers.

The combinations of labels establish a code for identifying different detection probes and, by extension, different target molecules to which those detection probes are associated with. This labeling scheme is referred to as Combinatorial Multicolor Coding (CMC). Such coding is described by Speicher et al., Nature Genetics 12:368-375 (1996). Use of

30   CMC is described in U.S. Patent No. 6,143,495. Any number of labels, which when combined can be separately detected, can be used for combinatorial multicolor coding. It is preferred that 2, 3, 4, 5, or 6 labels be used in combination. It is most preferred that 6 labels be used. The number of labels used establishes the number of unique label combinations

that can be formed according to the formula $2^N-1$, where N is the number of labels. According to this formula, 2 labels forms three label combinations, 3 labels forms seven label combinations, 4 labels forms 15 label combinations, 5 labels form 31 label combinations, and 6 labels forms 63 label combinations.

5          For combinatorial multicolor coding, a group of different detection probes are used as a set. Each type of detection probe in the set is labeled with a specific and unique combination of fluorescent labels. For those detection probes assigned multiple labels, the labeling can be accomplished by labeling each detection probe molecule with all of the required labels. Alternatively, pools of detection probes of a given type can each be labeled

10        with one of the required labels. By combining the pools, the detection probes will, as a group, contain the combination of labels required for that type of detection probe. Where each detection probe is labeled with a single label, label combinations can also be generated by using primers with coded combinations of detection tags complementary to the different detection probes. In this scheme, the primers will contain a combination of detection tags

15        representing the combination of labels required for a specific label code. Further illustrations are described in U.S. Patent No. 6,143,495. Use of pools of detection probes each probe with a single label is preferred when fluorescent change probes are used.

Speicher *et al.* describes a set of fluors and corresponding optical filters spaced across the spectral interval 350-770 nm that give a high degree of discrimination between

20        all possible fluor pairs. This fluor set, which is preferred for combinatorial multicolor coding, consists of 4'-6-diamidino-2-phenylinodole (DAPI), fluorescein (FITC), and the cyanine dyes Cy3, Cy3.5, Cy5, Cy5.5 and Cy7. Any subset of this preferred set can also be used where fewer combinations are required. The absorption and emission maxima, respectively, for these fluors are: DAPI (350 nm; 456 nm), FITC (490 nm; 520 nm), Cy3

25        (554 nm; 568 nm), Cy3.5 (581 nm; 588 nm), Cy5 (652 nm; 672 nm), Cy5.5 (682 nm; 703 nm) and Cy7 (755 nm; 778 nm). The excitation and emission spectra, extinction coefficients and quantum yield of these fluors are described by Ernst *et al.*, *Cytometry* 10:3-10 (1989), Mujumdar *et al.*, *Cytometry* 10:11-19 (1989), Yu, *Nucleic Acids Res.* 22:3226-3232 (1994), and Waggoner, *Meth. Enzymology* 246:362-373 (1995). These fluors can all

30        be excited with a 75W Xenon arc.

To attain selectivity, filters with bandwidths in the range of 5 to 16 nm are preferred. To increase signal discrimination, the fluors can be both excited and detected at wavelengths far from their spectral maxima. Emission bandwidths can be made as wide as

possible. For low-noise detectors, such as cooled CCD cameras, restricting the excitation bandwidth has little effect on attainable signal to noise ratios. A list of preferred filters for use with the preferred fluor set is listed in Table 1 of Speicher *et al.* It is important to prevent infra-red light emitted by the arc lamp from reaching the detector; CCD chips are

5      extremely sensitive in this region. For this purpose, appropriate IR blocking filters can be inserted in the image path immediately in front of the CCD window to minimize loss of image quality. Image analysis software can then be used to count and analyze the spectral signatures of fluorescent dots.

### i. Enzyme-linked Detection

10      Amplified nucleic acid labeled by incorporation of labeled nucleotides can be detected with established enzyme-linked detection systems. For example, amplified nucleic acid labeled by incorporation of biotin using biotin-16-UTP (Roche Molecular Biochemicals) can be detected as follows. The nucleic acid is immobilized on a solid glass surface by hybridization with a complementary DNA oligonucleotide (address probe)

15      complementary to the target sequence (or its complement) present in the amplified nucleic acid. After hybridization, the glass slide is washed and contacted with alkaline phosphatase-streptavidin conjugate (Tropix, Inc., Bedford, MA). This enzyme-streptavidin conjugate binds to the biotin moieties on the amplified nucleic acid. The slide is again washed to remove excess enzyme conjugate and the chemiluminescent substrate CSPD

20      (Tropix, Inc.) is added and covered with a glass cover slip. The slide can then be imaged in a Biorad Fluorimager.

### 3. Linear Strand Displacement Amplification

A modified form of multiple strand displacement amplification can be performed which results in linear amplification of a target sequence. This modified method is referred

25      to as linear strand displacement amplification (LSDA) and is accomplished by using a set of primers where all of the primers are complementary to the same strand of the target sequence. In LSDA, as in MSDA, the set of primers hybridize to the target sequence and strand displacement amplification takes place. However, only one of the strands of the target sequence is replicated. LSDA requires thermal cycling between each round of

30      replication to allow a new set of primers to hybridize to the target sequence. Such thermal cycling is similar to that used in PCR. Unlike linear, or single primer, PCR, however, each round of replication in LSDA results in multiple copies of the target sequence. One copy is

made for each primer used. Thus, if 20 primers are used in LSDA, 20 copies of the target sequence will be made in each cycle of replication.

DNA amplified using MSDA and WGSDA can be further amplified by transcription. For this purpose, promoter sequences can be included in the non-

5    complementary portion of primers used for strand displacement amplification, or in linker sequences used to concatenate DNA for MSDA-CD.

### 4. Reverse Transcription Multiple Displacement Amplification

Multiple displacement amplification can be performed on RNA or on DNA strands reverse transcribed from RNA. A useful form of the disclosed method, referred to as

10   reverse transcription multiple displacement amplification (RT-MDA) involves reverse transcribing RNA, removal of the RNA (preferably by nuclease digestion using an RNA-specific nuclease such as RNAse H), and multiple displacement amplification of the reverse transcribed DNA. RT-MDA can be performed using either double-stranded cDNA or using just the first cDNA strand. In the latter case, the second cDNA strand need not be, and

15   preferably is not, synthesized. RT-MDA is useful for quantitative analysis of mRNA or general amplification of mRNA sequences for any other purpose.

### 5. Repeat Multiple Displacement Amplification

The disclosed multiple displacement amplification operations can also be sequentially combined. For example, the product of MDA can itself be amplified in another

20   multiple displacement amplification. This is referred to herein as repeat multiple displacement amplification (RMDA). This can be accomplished, for example, by diluting the replicated strands following MDA and subjecting them to a new MDA. This can be repeated one or more times. Each round of MDA will increase the amplification. Different forms of MDA, such as WGSDA and MSDA on particular target sequences can be

25   combined. In general, repeat MDA can be accomplished by first bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. Replication of the target sequence results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication

30   of another replicated strand; and then diluting the replicated strands, bringing into contact a set of primers, DNA polymerase, and the diluted replicated strands, and incubating the replicated strands under conditions that promote replication of the target sequence. Replication of the target sequence results in additional replicated strands, wherein during

replication at least one of the additional replicated strands is displaced from the target sequence by strand displacement replication of another additional replicated strand. This form of the method can be extended by performing the following operation one or more times: diluting the additional replicated strands, bringing into contact a set of primers, DNA

5      polymerase, and the diluted replicated strands, and incubating the replicated strands under conditions that promote replication of the target sequence. Replication of the target sequence results in additional replicated strands, wherein during replication at least one of the additional replicated strands is displaced from the target sequence by strand displacement replication of another additional replicated strand.

10        **6.  Using Products of Multiple Displacement Amplification**

          The nucleic acids produced using the disclosed method can be used for any purpose. For example, the amplified nucleic acids can be analyzed (such as by sequencing or probe hybridization) to determine characteristics of the amplified sequences or the presence or absence or certain sequences. The amplified nucleic acids can also be used as reagents for

15     assays or other methods. For example, nucleic acids produced in the disclosed method can be coupled or adhered to a solid-state substrate. The resulting immobilized nucleic acids can be used as probes or indexes of sequences in a sample. Nucleic acids produced in the disclosed method can be coupled or adhered to a solid-state substrate in any suitable way. For example, nucleic acids generated by multiple strand displacement can be attached by

20     adding modified nucleotides to the 3' ends of nucleic acids produced by strand displacement replication using terminal deoxynucleotidyl transferase, and reacting the modified nucleotides with a solid-state substrate or support thereby attaching the nucleic acids to the solid-state substrate or support.

          Nucleic acids produced in the disclosed method also can be used as probes or

25     hybridization partners. For example, sequences of interest can be amplified in the disclosed method and provide a ready source of probes. The replicated strands (produced in the disclosed method) can be cleaved prior to use as hybridization probes. For example, the replicated strands can be cleaved with DNAse I. The hybridization probes can be labeled as described elsewhere herein with respect to labeling of nucleic acids produce in the disclosed

30     method.

          Nucleic acids produced in the disclosed method also can be used for subtractive hybridization to identify sequences that are present in only one of a pair or set of samples. For example, amplified cDNA from different samples can be annealed and the resulting

double-stranded material can be separated from single-stranded material. Unhybridized sequences would be indicative of sequences expressed in one of the samples but not others.

**Specific Embodiments**

Disclosed is a method of amplifying genomes, the method comprising, bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample. The primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the genomic nucleic acid sample.

The genome can be a eukaryotic genome, a plant genome, an animal genome, a vertebrate genome, a fish genome, a mammalian genome, a human genome, a microbial genome or a viral genome. The amplification bias can be less than 20-fold for at least ten nucleic acid sequences in the genomic nucleic acid sample. The amplification bias can be less than 10-fold for at least ten nucleic acid sequences in the genomic nucleic acid sample. The primer has a length of 3 nucleotides, 4 nucleotides, 5 nucleotides, 6 nucleotides, 7 nucleotides, 8 nucleotides, 9 nucleotides, 10 nucleotides, 11 nucleotides, 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides.

The primer can have a length of less than 4 nucleotides, less than 5 nucleotides, less than 6 nucleotides, less than 7 nucleotides, less than 8 nucleotides, less than 9 nucleotides, less than 10 nucleotides, less than 11 nucleotides, less than 12 nucleotides, less than 13 nucleotides, less than 14 nucleotides, less than 15 nucleotides, less than 16 nucleotides, less than 17 nucleotides, less than 18 nucleotides, less than 19 nucleotides, less than 20 nucleotides, less than 21 nucleotides, less than 22 nucleotides, less than 23 nucleotides, less than 24 nucleotides, less than 25 nucleotides, less than 26 nucleotides, less than 27 nucleotides, less than 28 nucleotides, less than 29 nucleotides, less than 30 nucleotides, or less than 31 nucleotides.

200

The genomic nucleic acid sample can be incubated at 20°C, 21°C, 22°C, 23°C, 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, 34°C, 35°C, 36°C, 37°C, 38°C, 39°C, 40°C, 41°C, 42°C, 43°C, 44°C, 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C,

5      66°C, 67°C, 68°C, 69°C, 70°C, 71°C, 72°C, 73°C, 74°C, 75°C, 76°C, 77°C, 78°C, 79°C, or 80°C.

The genomic nucleic acid sample can be incubated at less than 21°C, less than 22°C, less than 23°C, less than 24°C, less than 25°C, less than 26°C, less than 27°C, less than 28°C, less than 29°C, less than 30°C, less than 31°C, less than 32°C, less than 33°C, less

10     than 34°C, less than 35°C, less than 36°C, less than 37°C, less than 38°C, less than 39°C, less than 40°C, less than 41°C, less than 42°C, less than 43°C, less than 44°C, less than 45°C, less than 46°C, less than 47°C, less than 48°C, less than 49°C, less than 50°C, less than 51°C, less than 52°C, less than 53°C, less than 54°C, less than 55°C, less than 56°C, less than 57°C, less than 58°C, less than 59°C, less than 60°C, less than 61°C, less than

15     62°C, less than 63°C, less than 64°C, less than 65°C, less than 66°C, less than 67°C, less than 68°C, less than 69°C, less than 70°C, less than 71°C, less than 72°C, less than 73°C, less than 74°C, less than 75°C, less than 76°C, less than 77°C, less than 78°C, less than 79°C, or less than 80°C.

The genomic nucleic acid sample can have a sequence complexity of at least $1 \times 10^3$

20     nucleotides, the genomic nucleic acid sample can have a sequence complexity of at least $1 \times 10^4$ nucleotides, the genomic nucleic acid sample can have a sequence complexity of at least $1 \times 10^5$ nucleotides, the genomic nucleic acid sample can have a sequence complexity of at least $1 \times 10^6$ nucleotides, the genomic nucleic acid sample can have a sequence complexity of at least $1 \times 10^7$ nucleotides, the genomic nucleic acid sample can have a

25     sequence complexity of at least $1 \times 10^8$ nucleotides, or the genomic nucleic acid sample can have a sequence complexity of at least $1 \times 10^9$ nucleotides.

The primer, DNA polymerase and genomic nucleic acid sample are brought into contact with 1 additional primer, with 2 additional primers, with 3 additional primers, with 4 additional primers, with 5 additional primers, with 6 additional primers, with 7 additional

30     primers, with 8 additional primers, with 9 additional primers, with 10 additional primers, with 11 additional primers, with 12 additional primers, with 13 additional primers, with 14 additional primers, with 15 additional primers, with 16 additional primers, with 17 additional primers, with 18 additional primers, with 19 additional primers, with 20

additional primers, with 21 additional primers, with 22 additional primers, with 23 additional primers, with 24 additional primers, with 25 additional primers, with 26 additional primers, with 27 additional primers, with 28 additional primers, with 29 additional primers, with 30 additional primers, with 31 additional primers, with 32

5    additional primers, with 33 additional primers, with 34 additional primers, with 35 additional primers, with 36 additional primers, with 37 additional primers, with 38 additional primers, with 39 additional primers, with 40 additional primers, with 41 additional primers, with 42 additional primers, with 43 additional primers, with 44 additional primers, with 45 additional primers, with 46 additional primers, with 47

10   additional primers, with 48 additional primers, with 49 additional primers, with 50 additional primers, with 51 additional primers, with 52 additional primers, with 53 additional primers, with 54 additional primers, with 55 additional primers, with 56 additional primers, with 57 additional primers, with 58 additional primers, with 59 additional primers, with 60 additional primers, with 61 additional primers, with 62

15   additional primers, with 63 additional primers, with 75 additional primers, with 100 additional primers, with 150 additional primers, with 200 additional primers, with 300 additional primers, with 400 additional primers, with 500 additional primers, with 750 additional primers, or with 1,000 additional primers, wherein each primer can have a different specific nucleotide sequence.

20           The primers are all of the same length.

            The primer, DNA polymerase and genomic nucleic acid sample are brought into contact with fewer than 2 additional primers, with fewer than 3 additional primers, with fewer than 4 additional primers, with fewer than 5 additional primers, with fewer than 6 additional primers, with fewer than 7 additional primers, with fewer than 8 additional

25   primers, with fewer than 9 additional primers, with fewer than 10 additional primers, with fewer than 11 additional primers, with fewer than 12 additional primers, with fewer than 13 additional primers, with fewer than 14 additional primers, with fewer than 15 additional primers, with fewer than 16 additional primers, with fewer than 17 additional primers, with fewer than 18 additional primers, with fewer than 19 additional primers, with fewer than 20

30   additional primers, with fewer than 21 additional primers, with fewer than 22 additional primers, with fewer than 23 additional primers, with fewer than 24 additional primers, with fewer than 25 additional primers, with fewer than 26 additional primers, with fewer than 27 additional primers, with fewer than 28 additional primers, with fewer than 29 additional

primers, with fewer than 30 additional primers, with fewer than 31 additional primers, with fewer than 32 additional primers, with fewer than 33 additional primers, with fewer than 34 additional primers, with fewer than 35 additional primers, with fewer than 36 additional primers, with fewer than 37 additional primers, with fewer than 38 additional primers, with

5     fewer than 39 additional primers, with fewer than 40 additional primers, with fewer than 41 additional primers, with fewer than 42 additional primers, with fewer than 43 additional primers, with fewer than 44 additional primers, with fewer than 45 additional primers, with fewer than 46 additional primers, with fewer than 47 additional primers, with fewer than 48 additional primers, with fewer than 49 additional primers, with fewer than 50 additional

10     primers, with fewer than 51 additional primers, with fewer than 52 additional primers, with fewer than 53 additional primers, with fewer than 54 additional primers, with fewer than 55 additional primers, with fewer than 56 additional primers, with fewer than 57 additional primers, with fewer than 58 additional primers, with fewer than 59 additional primers, with fewer than 60 additional primers, with fewer than 61 additional primers, with fewer than 62

15     additional primers, with fewer than 63 additional primers, with fewer than 64 additional primers, with fewer than 75 additional primers, with fewer than 100 additional primers, with fewer than 150 additional primers, with fewer than 200 additional primers, with fewer than 300 additional primers, with fewer than 400 additional primers, with fewer than 500 additional primers, with fewer than 750 additional primers, or with fewer than 1,000

20     additional primers, wherein each primer can have a different specific nucleotide sequence.

Each primer can have a different one of the sequences AGTGGG or AGAGAG. Each primer can have a different one of the sequences AGCCGG, AGTAGG, or AGTTGG. Each primer can have a different one of the sequences AGGCGG, AGTGGG, AGGGAG, or AGTGAG. Each primer can have a different one of the sequences AGTGGG,

25     AGCCAG, AGTTAG, AGTCAG, or AGACAG. Each primer can have a different one of the sequences AGAGGG, AGGCAG, AGCCAG, AGTCAG, or AGACAG. Each primer can have a different one of the sequences AGTAGG, AGGTGG, AGGCAG, AGACAG, or AGTGAG. Each primer can have a different one of the sequences AGGAGG, AGAGGG, AGGGAG, AGTCAG, or AGCGAG. Each primer can have a different one of the

30     sequences CGGTGG, TCACGC, CGAGCG, GCGTGG, ACTCGG, AATCGC, CGGAGG, CCGAGA, GATCGC, AGAGCG, AGCGAG, or ACTCCG.

Each primer can have one of the sequences AGTGGG or AGAGAG. Each primer can have one of the sequences AGCCGG, AGTAGG, or AGTTGG. Each primer can have

203

one of the sequences AGGCGG, AGTGGG, AGGGAG, or AGTGAG. Each primer can have one of the sequences AGTGGG, AGCCAG, AGTTAG, AGTCAG, or AGACAG. Each primer can have one of the sequences AGAGGG, AGGCAG, AGCCAG, AGTCAG, or AGACAG. Each primer can have one of the sequences CGGTGG, TCACGC,

5      CGAGCG, GCGTGG, ACTCGG, AATCGC, CGGAGG, CCGAGA, GATCGC, AGAGCG, AGCGAG, or ACTCCG.

The primer can be complementary to a sequence in a repeat sequence.

The repeat sequence can be a microsatellite sequence, a minisatellite sequence, a satellite sequence, a transposon sequence, a ribosomal RNA sequence, a short interspersed

10     nuclear element (SINE), or a long interspersed nuclear element (LINE). The primer can be complementary to a sequence in a functional consensus sequence. The functional consensus sequence can be a promoter sequence, an enhancer sequence, a silencer sequence, an upstream regulatory element sequence, a transcription termination site sequence, a transposon regulatory sequence, a ribosomal RNA regulatory sequence, or a

15     polyadenylation site sequence. The functional consensus sequence can be a microbial promoter sequence, a microbial enhancer sequence, a microbial silencer sequence, a microbial upstream regulatory element sequence, a microbial transcription termination site sequence, a microbial transposon regulatory sequence, a microbial ribosomal RNA regulatory sequence, or a microbial polyadenylation site sequence.

20     The primer can be a broad coverage primer. The primer can be complementary to a sequence that occurs every 5,000 nucleotides or less, every 4,000 nucleotides or less, every 3,000 nucleotides or less, every 2,500 nucleotides or less, every 2,000 nucleotides or less, every 1,500 nucleotides or less, every 1,000 nucleotides or less, every 900 nucleotides or less, every 800 nucleotides or less, every 700 nucleotides or less, every 600 nucleotides or

25     less, every 500 nucleotides or less, every 400 nucleotides or less, every 300 nucleotides or less, every 200 nucleotides or less, every 100 nucleotides or less, or every 50 nucleotides or less, on average, in the nucleic acid molecules of the genomic nucleic acid sample.

The primer can have a G+C percentage within 20%, within 15%, within 10%, within 9%, within 8%, within 7%, within 6%, within 5%, within 4%, within 3%, within 2%, or

30     within 1% of the G+C percentage of the genomic nucleic acid sample. The primer produces a locus representation of at least 10% for at least 5 different loci for the type of genomic nucleic acid sample used. The primer produces a locus representation of at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%,

at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% for at least 5 different loci for the type of genomic nucleic acid sample used. The primer produces a locus representation of at least 10% for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at

5       least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci for the type of genomic nucleic acid sample used.

10      The primer produces an amplification bias of less than 50-fold for the type of genomic nucleic acid sample used. The primer produces an amplification bias of less than 45-fold, less than 40-fold, less than 35-fold, less than 30-fold, less than 25-fold, less than 20-fold, less than 19-fold, less than 18-fold, less than 17-fold, less than 16-fold, less than 15-fold, less than 14-fold, less than 13-fold, less than 12-fold, less than 11-fold, less than

15      10-fold, less than 9-fold, less than 8-fold, less than 7-fold, less than 6-fold, less than 5-fold, or less than 4-fold for the type of genomic nucleic acid sample used. The primer produces an amplification bias of less than 50-fold for at least 5 different loci, for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at

20      least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci for the type of genomic nucleic acid sample used.

25      The primer does not have an inter-complementary 3' end. The primer does not produce significant replication products in the absence of a nucleic acid sample. The DNA polymerase can be φ29 DNA polymerase. The genomic nucleic acid sample need not be subjected to denaturing conditions. The genomic nucleic acid sample need not be subjected to heat denaturing conditions. The genomic nucleic acid sample need not be subjected to

30      alkaline denaturing conditions. The genomic nucleic acid sample can be subjected to denaturing conditions. The genomic nucleic acid sample can be subjected to heat denaturing conditions. The genomic nucleic acid sample can be subjected to alkaline denaturing conditions.

Nucleic acids in the genomic nucleic acid sample are not separated from other material in the genomic nucleic acid sample. The genomic nucleic acid sample can be a crude cell lysate. The genomic nucleic acid sample can be produced by exposing cells to alkaline conditions to form a cell lysate, wherein the cell lysate can comprise a whole genome, and reducing the pH of the cell lysate to form a stabilized cell lysate. The cells are exposed to alkaline conditions by mixing the cells with a lysis solution. The lysis solution can comprise a base. The pH of the cell lysate can be reduced by mixing the cell lysate with a stabilization solution. The stabilization solution can comprise a buffer. The stabilization solution can comprise an acid.

Nucleic acids in the cell lysate and the stabilized cell lysate are not separated from other material in the cell lysate. The cell lysate and the stabilized cell lysate are not subjected to purification prior to the incubation. The cell lysate, stabilized cell lysate, or both are subjected to partial purification prior to the incubation. The cell lysate and the stabilized cell lysate are not subjected to substantial purification prior to the incubation. The incubation can be substantially isothermic. Neither the cell lysate nor the stabilized cell lysate can be heated substantially above the temperature of the incubation. Neither the cell lysate nor the stabilized cell lysate can be subjected to substantial heating above the temperature of the incubation. The cells are not heated substantially above the temperature of the incubation. The cells are not subjected to substantial heating above the temperature of the incubation. The cells are not heated substantially above the temperature at which the cells grow. The cells are not subjected to substantial heating above the temperature at which the cells grow.

Neither the cell lysate nor the stabilized cell lysate can be heated above a temperature and for a time that would cause notable denaturation of the genome. Neither the cell lysate nor the stabilized cell lysate can be subjected to heating above a temperature and for a time that would cause notable denaturation of the genome. The cells are not lysed by heat. The cells are not heated above a temperature and for a time that would cause substantial cell lysis in the absence of the alkaline conditions. The cells are not subjected to heating above a temperature and for a time that would cause substantial cell lysis in the absence of the alkaline conditions.

The method can further comprise, prior to bringing into contact the primer, the genomic nucleic acid sample and the DNA polymerase, exposing the genomic nucleic acid sample to conditions that promote substantial denaturation of the nucleic acid molecules in

206

the genomic nucleic acid sample, thereby forming a denatured genomic nucleic acid sample, and altering the conditions to conditions that do not promote substantial denaturation of nucleic acid molecules in the genomic nucleic acid sample to form a denatured genomic nucleic acid sample.

5      Replication of the nucleic acid molecules in the genomic nucleic acid sample results in a longer average fragment length for the replicated nucleic acid molecules than the average fragment length in the genomic nucleic acid sample. The genomic nucleic acid sample, the denatured genomic nucleic acid sample, or both are exposed to ionic conditions. The genomic nucleic acid sample can be exposed to conditions that promote substantial

10     denaturation by mixing the genomic nucleic acid sample with a denaturing solution and by heating the genomic nucleic acid sample to a temperature and for a length of time that substantially denatures the nucleic acid molecules in the genomic nucleic acid sample.

The primer contains at least one modified nucleotide such that the primer can be resistant to 3'-5' exonuclease. The primer can be 6 nucleotides long, wherein the primer

15     contains at least one modified nucleotide such that the primer can be nuclease resistant, and wherein the DNA polymerase can be $\phi$29 DNA polymerase. The conditions that promote replication of the nucleic acid molecules are substantially isothermic. The conditions that promote replication of the nucleic acid molecules do not involve thermal cycling. The conditions that promote replication of the nucleic acid molecules do not include thermal

20     cycling. The primer can comprise nucleotides, wherein one or more of the nucleotides are ribonucleotides. From about 10% to about 50% of the nucleotides are ribonucleotides. About 50% or more of the nucleotides are ribonucleotides. All of the nucleotides are ribonucleotides.

The primer can comprise nucleotides, wherein one or more of the nucleotides are 2'-

25     O-methyl ribonucleotides. From about 10% to about 50% of the nucleotides are 2'-O-methyl ribonucleotides. About 50% or more of the nucleotides are 2'-O-methyl ribonucleotides. All of the nucleotides are 2'-O-methyl ribonucleotides. The primer can comprise nucleotides, wherein the nucleotides are a mixture of ribonucleotides and 2'-O-methyl ribonucleotides. The primer can comprise nucleotides, wherein the nucleotides are a

30     mixture of deoxyribonucleotides and 2'-O-methyl ribonucleotides. The genomic nucleic acid sample can be a blood sample, a urine sample, a semen sample, a lymphatic fluid sample, a cerebrospinal fluid sample, amniotic fluid sample, a biopsy sample, a needle aspiration biopsy sample, a cancer sample, a tumor sample, a tissue sample, a cell sample, a

cell lysate sample, a crude cell lysate sample, a forensic sample, an archeological sample, an infection sample, a nosocomial infection sample, a production sample, a drug preparation sample, a biological molecule production sample, a protein preparation sample, a lipid preparation sample, a carbohydrate preparation sample, or a combination thereof.

5        The genomic nucleic acid sample can be a crude cell lysate. The genomic nucleic acid sample need not be processed beyond cell lysis. The replicated nucleic acid molecules are analyzed. The replicated nucleic acid molecules are analyzed using one or more DNA chips. The replicated nucleic acid molecules are analyzed by hybridization. The replicated nucleic acid molecules are analyzed by nucleic acid sequencing. The replicated nucleic acid
10      molecules are stored prior to, following, or both prior to and following their analysis.

         The method can further comprise bringing into contact the primer, DNA polymerase, and a second genomic nucleic acid sample, and incubating the second genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the second genomic nucleic acid sample, wherein the second genomic nucleic acid sample
15      can comprise all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the second genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the second genomic nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the second genomic nucleic acid sample.

20      The second genomic nucleic acid sample can be a sample from the same type of organism as the first genomic nucleic acid sample. The second genomic nucleic acid sample can be a sample from the same type of tissue as the first genomic nucleic acid sample. The second genomic nucleic acid sample can be a sample from the same organism as the first genomic nucleic acid sample. The second genomic nucleic acid sample can be
25      obtained at a different time than the first genomic nucleic acid sample. The second genomic nucleic acid sample can be a sample from a different organism than the first genomic nucleic acid sample. The second genomic nucleic acid sample can be a sample from a different type of tissue than the first genomic nucleic acid sample.

         The second genomic nucleic acid sample can be a sample from a different species of
30      organism than the first genomic nucleic acid sample. The second genomic nucleic acid sample can be a sample from a different strain of organism than the first genomic nucleic acid sample. The second genomic nucleic acid sample can be a sample from a different cellular compartment than the first genomic nucleic acid sample.

Also disclosed is a method of amplifying genomes, the method comprising, bringing into contact fewer than 1,000 primers, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample, wherein each

5    primer has a different specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in

10   the genomic nucleic acid sample.

The DNA polymerase and genomic nucleic acid sample are brought into contact with fewer than 2 primers, with fewer than 3 primers, with fewer than 4 primers, with fewer than 5 primers, with fewer than 6 primers, with fewer than 7 primers, with fewer than 8 primers, with fewer than 9 primers, with fewer than 10 primers, with fewer than 11 primers,

15   with fewer than 12 primers, with fewer than 13 primers, with fewer than 14 primers, with fewer than 15 primers, with fewer than 16 primers, with fewer than 17 primers, with fewer than 18 primers, with fewer than 19 primers, with fewer than 20 primers, with fewer than 21 primers, with fewer than 22 primers, with fewer than 23 primers, with fewer than 24 primers, with fewer than 25 primers, with fewer than 26 primers, with fewer than 27

20   primers, with fewer than 28 primers, with fewer than 29 primers, with fewer than 30 primers, with fewer than 31 primers, with fewer than 32 primers, with fewer than 33 primers, with fewer than 34 primers, with fewer than 35 primers, with fewer than 36 primers, with fewer than 37 primers, with fewer than 38 primers, with fewer than 39 primers, with fewer than 40 primers, with fewer than 41 primers, with fewer than 42

25   primers, with fewer than 43 primers, with fewer than 44 primers, with fewer than 45 primers, with fewer than 46 primers, with fewer than 47 primers, with fewer than 48 primers, with fewer than 49 primers, with fewer than 50 primers, with fewer than 51 primers, with fewer than 52 primers, with fewer than 53 primers, with fewer than 54 primers, with fewer than 55 primers, with fewer than 56 primers, with fewer than 57

30   primers, with fewer than 58 primers, with fewer than 59 primers, with fewer than 60 primers, with fewer than 61 primers, with fewer than 62 primers, with fewer than 63 primers, with fewer than 64 primers, with fewer than 75 primers, with fewer than 100 primers, with fewer than 150 primers, with fewer than 200 primers, with fewer than 300

primers, with fewer than 400 primers, with fewer than 500 primers, with fewer than 750 primers, or with fewer than 1,000 primers.

Also disclosed is a method of amplifying nucleic acid samples of notable sequence complexity, the method comprising, bringing into contact a single primer, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under conditions that promote replication of nucleic acid molecules in the nucleic acid sample, wherein the primer has a specific nucleotide sequence, wherein the nucleic acid sample has a sequence complexity of at least $1 \times 10^4$ nucleotides, wherein replication of nucleic acid molecules in the nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the nucleic acid sample.

The nucleic acid sample can have a sequence complexity of at least $1 \times 10^5$ nucleotides, the nucleic acid sample can have a sequence complexity of at least $1 \times 10^6$ nucleotides, the nucleic acid sample can have a sequence complexity of at least $1 \times 10^7$ nucleotides, the nucleic acid sample can have a sequence complexity of at least $1 \times 10^8$ nucleotides, or the nucleic acid sample can have a sequence complexity of at least $1 \times 10^9$ nucleotides. The nucleic acid sample is or is derived from a genome, a chromosome, a chromosome fragment, an artificial chromosome, a yeast artificial chromosome, a bacterial artificial chromosome, a cosmid, or a combination.

The nucleic acid sample is or is derived from a blood sample, a urine sample, a semen sample, a lymphatic fluid sample, a cerebrospinal fluid sample, amniotic fluid sample, a biopsy sample, a needle aspiration biopsy sample, a cancer sample, a tumor sample, a tissue sample, a cell sample, a cell lysate sample, a crude cell lysate sample, a forensic sample, an archeological sample, an infection sample, a nosocomial infection sample, a production sample, a drug preparation sample, a biological molecule production sample, a protein preparation sample, a lipid preparation sample, a carbohydrate preparation sample, or a combination thereof. The nucleic acid sample is or is derived from a eukaryote, a plant, and animal, a marine animal, a vertebrate, a mammal, or a human.

Also disclosed is a method of amplifying genomes, the method comprising, bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample, wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a

substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein the genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^9$ nucleotides, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results

5        in replication of at least 0.01% of the nucleic acid sequences in the genomic nucleic acid sample.

Replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 0.1% of the nucleic acid sequences in the genomic nucleic acid sample, at least 1% of the nucleic acid sequences in the genomic nucleic acid sample, at

10      least 5% of the nucleic acid sequences in the genomic nucleic acid sample, at least 10% of the nucleic acid sequences in the genomic nucleic acid sample, at least 20% of the nucleic acid sequences in the genomic nucleic acid sample, at least 30% of the nucleic acid sequences in the genomic nucleic acid sample, at least 40% of the nucleic acid sequences in the genomic nucleic acid sample, at least 50% of the nucleic acid sequences in the genomic

15      nucleic acid sample, at least 60% of the nucleic acid sequences in the genomic nucleic acid sample, at least 70% of the nucleic acid sequences in the genomic nucleic acid sample, at least 80% of the nucleic acid sequences in the genomic nucleic acid sample, at least 90% of the nucleic acid sequences in the genomic nucleic acid sample, at least 95% of the nucleic acid sequences in the genomic nucleic acid sample, at least 96% of the nucleic acid

20      sequences in the genomic nucleic acid sample, at least 97% of the nucleic acid sequences in the genomic nucleic acid sample, at least 98% of the nucleic acid sequences in the genomic nucleic acid sample, or at least 99% of the nucleic acid sequences in the genomic nucleic acid sample.

Also disclosed is a method of amplifying genomes, the method comprising, bringing

25      into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample, wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the

30      genomic nucleic acid sample proceeds by strand displacement replication, wherein the genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^8$ nucleotides, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results

in replication of at least 0.1% of the nucleic acid sequences in the genomic nucleic acid sample.

Replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 1% of the nucleic acid sequences in the genomic nucleic acid

5      sample, at least 5% of the nucleic acid sequences in the genomic nucleic acid sample, at least 10% of the nucleic acid sequences in the genomic nucleic acid sample, at least 20% of the nucleic acid sequences in the genomic nucleic acid sample, at least 30% of the nucleic acid sequences in the genomic nucleic acid sample, at least 40% of the nucleic acid sequences in the genomic nucleic acid sample, at least 50% of the nucleic acid sequences in

10     the genomic nucleic acid sample, at least 60% of the nucleic acid sequences in the genomic nucleic acid sample, at least 70% of the nucleic acid sequences in the genomic nucleic acid sample, at least 80% of the nucleic acid sequences in the genomic nucleic acid sample, at least 90% of the nucleic acid sequences in the genomic nucleic acid sample, at least 95% of the nucleic acid sequences in the genomic nucleic acid sample, at least 96% of the nucleic

15     acid sequences in the genomic nucleic acid sample, at least 97% of the nucleic acid sequences in the genomic nucleic acid sample, at least 98% of the nucleic acid sequences in the genomic nucleic acid sample, or at least 99% of the nucleic acid sequences in the genomic nucleic acid sample.

Also disclosed is a method of amplifying genomes, the method comprising, bringing

20     into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample, wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the

25     genomic nucleic acid sample proceeds by strand displacement replication, wherein the genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^7$ nucleotides, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 1% of the nucleic acid sequences in the genomic nucleic acid sample.

30     Replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 5% of the nucleic acid sequences in the genomic nucleic acid sample, at least 10% of the nucleic acid sequences in the genomic nucleic acid sample, at least 20% of the nucleic acid sequences in the genomic nucleic acid sample, at least 30% of

the nucleic acid sequences in the genomic nucleic acid sample, at least 40% of the nucleic acid sequences in the genomic nucleic acid sample, at least 50% of the nucleic acid sequences in the genomic nucleic acid sample, at least 60% of the nucleic acid sequences in the genomic nucleic acid sample, at least 70% of the nucleic acid sequences in the genomic

5    nucleic acid sample, at least 80% of the nucleic acid sequences in the genomic nucleic acid sample, at least 90% of the nucleic acid sequences in the genomic nucleic acid sample, at least 95% of the nucleic acid sequences in the genomic nucleic acid sample, at least 96% of the nucleic acid sequences in the genomic nucleic acid sample, at least 97% of the nucleic acid sequences in the genomic nucleic acid sample, at least 98% of the nucleic acid

10   sequences in the genomic nucleic acid sample, or at least 99% of the nucleic acid sequences in the genomic nucleic acid sample.

Also disclosed is a method of amplifying genomes, the method comprising, bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of

15   nucleic acid molecules in the genomic nucleic acid sample, wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein the genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^6$ nucleotides,

20   wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 10% of the nucleic acid sequences in the genomic nucleic acid sample.

Replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 20% of the nucleic acid sequences in the genomic nucleic acid

25   sample, at least 30% of the nucleic acid sequences in the genomic nucleic acid sample, at least 40% of the nucleic acid sequences in the genomic nucleic acid sample, at least 50% of the nucleic acid sequences in the genomic nucleic acid sample, at least 60% of the nucleic acid sequences in the genomic nucleic acid sample, at least 70% of the nucleic acid sequences in the genomic nucleic acid sample, at least 80% of the nucleic acid sequences in

30   the genomic nucleic acid sample, at least 90% of the nucleic acid sequences in the genomic nucleic acid sample, at least 95% of the nucleic acid sequences in the genomic nucleic acid sample, at least 96% of the nucleic acid sequences in the genomic nucleic acid sample, at least 97% of the nucleic acid sequences in the genomic nucleic acid sample, at least 98% of

the nucleic acid sequences in the genomic nucleic acid sample, or at least 99% of the nucleic acid sequences in the genomic nucleic acid sample.

Also disclosed is a method of amplifying genomes, the method comprising, bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and

5      incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample, wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein the

10     genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^5$ nucleotides, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 80% of the nucleic acid sequences in the genomic nucleic acid sample.

Replication of the nucleic acid molecules in the genomic nucleic acid sample results

15     in replication of at least 90% of the nucleic acid sequences in the genomic nucleic acid sample, at least 95% of the nucleic acid sequences in the genomic nucleic acid sample, at least 96% of the nucleic acid sequences in the genomic nucleic acid sample, at least 97% of the nucleic acid sequences in the genomic nucleic acid sample, at least 98% of the nucleic acid sequences in the genomic nucleic acid sample, or at least 99% of the nucleic acid

20     sequences in the genomic nucleic acid sample.

Also disclosed is a method of amplifying genomes, the method comprising, bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample, wherein the primer has a

25     specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in a locus representation of at least 10% for at least 5 different loci. Replication of the nucleic

30     acid molecules in the genomic nucleic acid sample results in a locus representation of at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% for at least 5 different loci.

214

Replication of the nucleic acid molecules in the genomic nucleic acid sample results in a locus representation of at least 10% for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

Replication of the nucleic acid molecules in the genomic nucleic acid sample results in an amplification bias of less than 50-fold. Replication of the nucleic acid molecules in the genomic nucleic acid sample results in an amplification bias of less than 45-fold, less than 40-fold, less than 35-fold, less than 30-fold, less than 25-fold, less than 20-fold, less than 19-fold, less than 18-fold, less than 17-fold, less than 16-fold, less than 15-fold, less than 14-fold, less than 13-fold, less than 12-fold, less than 11-fold, less than 10-fold, less than 9-fold, less than 8-fold, less than 7-fold, less than 6-fold, less than 5-fold, or less than 4-fold.

Replication of the nucleic acid molecules in the genomic nucleic acid sample results in an amplification bias of less than 50-fold for at least 5 different loci, for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

Also disclosed is a method of amplifying nucleic acid samples of high sequence complexity, the method comprising, bringing into contact a single primer, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under conditions that promote replication of nucleic acid molecules in the nucleic acid sample, wherein the primer has a specific nucleotide sequence, wherein the nucleic acid sample has a sequence complexity of at least $1 \times 10^3$ nucleotides, wherein replication of nucleic acid molecules in the nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the nucleic acid sample results in a sequence representation of at least 10% for at least 5 different target sequences.

Replication of the nucleic acid molecules in the nucleic acid sample results in a sequence representation of at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% for at least 5 different target sequences.

Replication of the nucleic acid molecules in the nucleic acid sample results in a sequence representation of at least 10% for at least 6 different target sequences, at least 7 different target sequences, at least 8 different target sequences, at least 9 different target sequences, at least 10 different target sequences, at least 11 different target sequences, at least 12 different target sequences, at least 13 different target sequences, at least 14 different target sequences, at least 15 different target sequences, at least 16 different target sequences, at least 17 different target sequences, at least 18 different target sequences, at least 19 different target sequences, at least 20 different target sequences, at least 25 different target sequences, at least 30 different target sequences, at least 40 different target sequences, at least 50 different target sequences, at least 75 different target sequences, or at least 100 different target sequences.

Replication of the nucleic acid molecules in the nucleic acid sample results in an amplification bias of less than 50-fold. Replication of the nucleic acid molecules in the nucleic acid sample results in an amplification bias of less than 45-fold, less than 40-fold, less than 35-fold, less than 30-fold, less than 25-fold, less than 20-fold, less than 19-fold, less than 18-fold, less than 17-fold, less than 16-fold, less than 15-fold, less than 14-fold, less than 13-fold, less than 12-fold, less than 11-fold, less than 10-fold, less than 9-fold, less than 8-fold, less than 7-fold, less than 6-fold, less than 5-fold, or less than 4-fold.

Replication of the nucleic acid molecules in the nucleic acid sample results in an amplification bias of less than 50-fold for at least 5 different target sequences, for at least 6 different target sequences, at least 7 different target sequences, at least 8 different target sequences, at least 9 different target sequences, at least 10 different target sequences, at least 11 different target sequences, at least 12 different target sequences, at least 13 different target sequences, at least 14 different target sequences, at least 15 different target sequences, at least 16 different target sequences, at least 17 different target sequences, at least 18 different target sequences, at least 19 different target sequences, at least 20 different target sequences, at least 25 different target sequences, at least 30 different target sequences, at least 40 different target sequences, at least 50 different target sequences, at least 75 different target sequences, or at least 100 different target sequences.

216

Also disclosed is a method of amplifying genomes, the method comprising, bringing into contact a set of primers, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample, wherein the set of primers

5    comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of all or a

10   substantial fraction of the nucleic acid molecules in the genomic nucleic acid sample, wherein each selected primer in the set can produce replication of at least 80% of the nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic

15   acid sample, wherein the selection nucleic acid sample has a sequence complexity of at least 1 X $10^8$ nucleotides.

The set of primers further can comprise at least one additional primer. The set of primers can further comprise at least one non-selected primer, wherein the non-selected primer produces replication of less than 80% of the nucleic acid sequences in a selection

20   nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample.

Also disclosed is a method of amplifying genomes, the method comprising, bringing into contact a set of primers, DNA polymerase, and a genomic nucleic acid sample, and

25   incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample, wherein the set of primers comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic

30   acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the genomic nucleic acid sample, wherein each selected primer in the set can produce an amplification bias of less than 20-

fold for at least 10 nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample, wherein the selection nucleic acid sample has a sequence

5       complexity of at least $1 \times 10^8$ nucleotides.

Also disclosed is a method of amplifying genomes, the method comprising, bringing into contact a set of primers, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample, wherein the set of primers

10      comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of all or a

15      substantial fraction of the nucleic acid molecules in the genomic nucleic acid sample, wherein each selected primer in the set can produce a sequence representation of at least 10% for at least 10 nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the

20      selection nucleic acid sample, wherein the selection nucleic acid sample has a sequence complexity of at least $1 \times 10^8$ nucleotides.

Also disclosed is a method of amplifying nucleic acids, the method comprising, bringing into contact a set of primers, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under conditions that promote replication of nucleic acid

25      molecules in the nucleic acid sample, wherein the set of primers comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein replication of nucleic acid molecules in the nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid

30      molecules in the nucleic acid sample, wherein each selected primer in the set can produce replication of at least 80% of the nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in

218

the selection nucleic acid sample, wherein the selection nucleic acid sample has a sequence complexity of at least $1 \times 10^8$ nucleotides.

The set of primers can further comprise at least one additional primer. The set of primers can further comprise at least one non-selected primer, wherein the non-selected primer produces replication of less than 80% of the nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample. The nucleic acid sample can have a sequence complexity of at least $1 \times 10^3$ nucleotides.

Also disclosed is a method of amplifying nucleic acids, the method comprising, bringing into contact a set of primers, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under conditions that promote replication of nucleic acid molecules in the nucleic acid sample, wherein the set of primers comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein replication of nucleic acid molecules in the nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the nucleic acid sample, wherein each selected primer in the set can produce an amplification bias of less than 20-fold for at least 10 nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample, wherein the selection nucleic acid sample has a sequence complexity of at least $1 \times 10^8$ nucleotides.

Also disclosed is a method of amplifying nucleic acids, the method comprising, bringing into contact a set of primers, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under conditions that promote replication of nucleic acid molecules in the nucleic acid sample, wherein the set of primers comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein replication of nucleic acid molecules in the nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the nucleic acid sample, wherein each selected primer in the set can produce a sequence representation of at least 10% for at least 10 nucleic acid sequences in a selection

219

nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample, wherein the selection nucleic acid sample has a sequence complexity of at least 1 X $10^8$ nucleotides.

5            Disclosed is a method of amplifying nucleic acids, the method comprising incubating nucleic acids comprising target sequences at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, and a set of primers, under conditions promoting replication of the nucleic acids. Replication of the nucleic acids results in replicated strands. During replication at least one

10       of the replicated nucleic acid strands is displaced by strand displacement replication of another replicated strand. Formation of replicated strands from the target sequences is favored over formation of replicated strands from non-target sequences.

            Also disclosed is a method of amplifying a whole genome, the method comprising exposing cells to alkaline conditions to form a cell lysate, reducing the pH of the cell lysate

15       to form a stabilized cell lysate, and incubating stabilized cell lysate at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, and a set of primers, under conditions promoting replication of the nucleic acids. Replication of the nucleic acids results in replicated strands. During replication at least one of the replicated nucleic acid strands is displaced by

20       strand displacement replication of another replicated strand. Formation of replicated strands from the target sequence is favored over formation of replicated strands from non-target sequences. The cell lysate comprises a whole genome.

            Also disclosed is a method of performing strand displacement nucleic acid synthesis at an elevated temperature, the method comprising mixing thermolabile nucleic acid

25       polymerase having strand-displacement activity, nucleic acids comprising target sequences, a set of primers, and an additive, and incubating at an elevated temperature and under conditions favoring hybridization of the primers to the target sequences and extension of the primers by the addition of nucleotides sequentially to the 3' end of the primer in a template-dependent manner, wherein the extension results in replication of the target sequences.

30       Also disclosed is a method of amplifying a whole genome, the method comprising exposing cells to alkaline conditions to form a cell lysate, wherein the cell lysate comprises a whole genome, reducing the pH of the cell lysate to form a stabilized cell lysate, and incubating stabilized cell lysate at an elevated temperature in the presence of a thermolabile

nucleic acid polymerase having strand displacement activity, an additive, a set of primers, and deoxyribonucleotide triphosphates under conditions promoting replication of nucleic acids. During replication at least one of the replicated nucleic acid strands is displaced by strand displacement replication of another replicated strand. Formation of template-

5      dependent extension products in the replication reaction is favored over formation of non-templated product.

       Also disclosed is a method of performing strand displacement nucleic acid synthesis at an elevated temperature, the method comprising mixing thermolabile nucleic acid polymerase having strand-displacement activity, single-stranded template nucleic acid, a set

10     of primers, deoxyribonucleotide triphosphates and an additive, and incubating at an elevated temperature and under conditions favoring hybridization of primer to template nucleic acid and extension of primer by the addition of nucleotides sequentially to the 3' end of the primer in a template-dependent manner, wherein said polymerization results in replication of said template nucleic acid.

15     Also disclosed is a method of amplifying nucleic acids, the method comprising incubating nucleic acids at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, a set of primers, and deoxyribonucleotide triphosphates under conditions promoting replication of nucleic acids. During replication at least one of the replicated nucleic acid strands is displaced by strand

20     displacement replication of another replicated strand. Formation of template-dependent extension products in the replication reaction is favored over formation of non-templated product.

       The elevated temperature can be a temperature at which the nucleic acid polymerase is substantially incapable of performing template-dependent polymerization in absence of

25     the additive. The elevated temperature can comprise a temperature at which said polymerase is substantially incapable of performing template-dependent polymerization in absence of said additive. The elevated temperature can be a temperature greater than 30 degrees Celsius. The elevated temperature can be a temperature greater than 32 degrees Celsius. The elevated temperature can be a temperature greater than 35 degrees Celsius.

30     The elevated temperature can be a temperature greater than 37 degrees Celsius. The thermolabile nucleic acid polymerase can be Phi29 DNA polymerase. The thermolabile nucleic acid polymerase can be Phi29 DNA polymerase, E.coli DNA polymerase, Bst large fragment DNA polymerase, Bca DNA polymerase, phage M2 DNA polymerase, phage

φPRD1 DNA polymerase, Klenow fragment of DNA polymerase I, T5 DNA polymerase, T4 DNA polymerase holoenzyme, or a combination.

The set of primers can comprise at least 2 primers. The set of primers can comprise at least 10 primers. The set of primers can comprise at least 50 primers. The set of primers can comprise greater than 200 primers. The set of primers can comprise greater than 1023 primers. The primers in the set of primers each can be 6 nucleotides in length. The primers in the set of primers each can be 8 nucleotides in length. The primers in the set of primers each can be longer than 8 nucleotides. Two or more of the primers in the set of primers can be of different lengths.

The additive can comprise a sugar or a combination of sugars. The additive can comprise trehalose, glucose, sucrose, or a combination. The additive can comprise a sugar, a chaperone, a protein, or a combination. Strand displacement replication is performed in presence of the additive. The additive can comprise trehalose. The incubation of the nucleic acids, nucleic acid polymerase, additive, and set of primers can be in the presence of deoxyribonucleotide triphosphates.

The ratio of replicated strands from target sequences to replicated strands from non-target sequences is less than the ratio of replicated strands from target sequences to replicated strands from non-target sequences if the nucleic acids are incubated in the presence of the same nucleic acid polymerase and set of primers and under the same conditions except not at an elevated temperature.

Also disclosed is a kit for amplifying nucleic acids, the kit comprising a thermolabile nucleic acid polymerase having strand displacement activity, an additive, and a set of primers, wherein incubating nucleic acids comprising target sequences at an elevated temperature in the presence of the thermolabile nucleic acid polymerase, the additive, and the set of primers under conditions promoting replication of the nucleic acids results in replicated strands and in formation of replicated strands from the target sequences in favor of formation of replicated strands from non-target sequences.

The elevated temperature can be a temperature at which the nucleic acid polymerase is substantially incapable of performing template-dependent polymerization in absence of the additive. The thermolabile nucleic acid polymerase, the additive, and the set of primers can be chosen such that incubating nucleic acids comprising target sequences at an elevated temperature in the presence of the thermolabile nucleic acid polymerase, the additive, and the set of primers under conditions promoting replication of the nucleic acids results in

222

replicated strands and in formation of replicated strands from the target sequences in favor of formation of replicated strands from non-target sequences.

The nucleic acid polymerase can be Phi29 DNA polymerase. The additive can be a sugar, a chaperone, a protein, trehalose, glucose, sucrose, or a combination. The additive can comprise trehalose, the set of primers can comprise exonuclease-resistant random hexamer primers, and the nucleic acid polymerase can comprise Phi29 DNA polymerase. The kit can further comprise one or more components that, when mixed in appropriate amounts, produce a reaction mixture having final concentrations of 10 mM MgCl$_2$, 37.5 mM Tris-HCl, pH 7, 50 mM KCl, 20 mM Ammonium Sulfate, and 1 mM dNTPs. The kit can further comprise any one or a combination of a stabilization solution, a lysis solution, a reaction mix that comprises the set of primers, dithiotheitol, Phosphate-Buffered Saline, and control DNA template. The stabilization solution can comprise 800 mM Tris-HCl, pH 4; the lysis solution can comprise 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA; the reaction mix can comprise 150 mM Tris-HCl, 200 mM KCl, 40 mM MgCl$_2$, 20 mM (NH$_4$)$_2$SO$_4$, 4 mM deoxynucleotide triphosphates, and 0.2 mM random hexamer primers; the dithiothreitol can comprise 1M dithiotheitol; and the Phosphate-Buffered Saline can comprise 1X Phosphate-Buffered Saline, pH 7.5.

Disclosed is a method of amplifying a target nucleic acid sequence where the method can comprise bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand. The target sample is not subjected to denaturing conditions. The method can further comprise labeling the replicated strands using terminal deoxynucleotidyl transferase.

The method can further comprise diluting the replicated strands, bringing into contact a set of primers, DNA polymerase, and the diluted replicated strands, and incubating the replicated strands under conditions that promote replication of the target sequence. Replication of the target sequence results in additional replicated strands, where during replication at least one of the additional replicated strands is displaced from the target sequence by strand displacement replication of another additional replicated strand. The method can further comprise performing the following operation one or more times: diluting the additional replicated strands, bringing into contact a set of primers, DNA

polymerase, and the diluted replicated strands, and incubating the replicated strands under conditions that promote replication of the target sequence. Replication of the target sequence results in additional replicated strands, where during replication at least one of the additional replicated strands is displaced from the target sequence by strand displacement replication of another additional replicated strand. The method can further comprise incubating the polymerase-target sample mixture under conditions that promote strand displacement. The method can further comprise bringing into contact a set of primers, DNA polymerase, and a second target sample, and incubating the second target sample under conditions that promote replication of the target sequence. The second target sample is not subjected to denaturing conditions. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

The primers can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides long. The primers can be 5, 6, 7, 8, 9, or 10 nucleotides long. The primers can be 5, 6, 7, or 8 nucleotides long. The primers can be 6, 7, or 8 nucleotides long. The primers can be 6 nucleotides long. The primers each can contain at least one modified nucleotide such that the primers are resistant to 3'-5' exonuclease. The DNA polymerase can be bacteriophage φ29 DNA polymerase, Tts DNA polymerase, phage M2 DNA polymerase, VENT™ DNA polymerase, Klenow fragment of DNA polymerase I, T5 DNA polymerase, PRD1 DNA polymerase, T4 DNA polymerase holoenzyme, T7 native polymerase T7 Sequenase®, or Bst DNA polymerase. The DNA polymerase can be φ29 DNA polymerase.

The primers can be 6 nucleotides long, the primers each can contain at least one modified nucleotide such that the primers are nuclease resistant, and the DNA polymerase can be φ29 DNA polymerase. The replicated strands can be labeled by the addition of modified nucleotides to the replicated strands. The modified nucleotides can be biotinylated nucleotides, fluorescent nucleotides, 5 methyl dCTP, BrdUTP, or 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphates. Modified nucleotides can be incorporated into the replicated strands during replication. The modified nucleotides can be biotinylated nucleotides, fluorescent nucleotides, 5 methyl dCTP, BrdUTP, or 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphates. The modified nucleotides can be 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphates and the replicated strands can be labeled by reacting labels with the incorporated 5-(3-aminoallyl)-2'-deoxyuridines. The labels can be fluorescein

224

isothiocyanate, 5,6-carboxymethyl fluorescein, Texas red, nitrobenz-2-oxa-1,3-diazol-4-yl, coumarin, dansyl chloride, rhodamine, amino-methyl coumarin, Eosin, Erythrosin, BODIPY®, Cascade Blue®, Oregon Green®, pyrene, lissamine, xanthene, acridine, oxazines, phycoerythrin, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, or a combination thereof.

5          The target sample need not be subjected to heat denaturing conditions. The target sequence can comprise two strands, and the set of primers can have 3 or more primers complementary to one of the strands of the target sequence and at least one primer complementary to the other strand of the target sequence. The set of primers can have 3 or more primers complementary to the same strand of the target sequence. The set of primers

10        can have 4 or more primers complementary to the same strand of the target sequence. The set of primers can have 4 or more primers. The set of primers can have 5 or more primers. The conditions that promote replication of the target sequence can be substantially isothermic. The conditions that promote replication of the target sequence need not involve thermal cycling. The conditions need not include thermal cycling. The target sequence can

15        comprise an amplification target and a hybridization target, the hybridization target can flank the amplification target, the set of primers can comprise a plurality of primers, each primer can comprise a complementary portion, where the complementary portions of the primers each can be complementary to a different portion of the hybridization target.

          The set of primers can comprise a right set of primers and a left set of primers, the

20        target sequence can be double-stranded, having a first and a second strand, the hybridization target can comprise a right and left hybridization target, where the right hybridization target can flank the amplification target on one end and the left hybridization target can flank the amplification target on the other end. The complementary portions of the right set primers can be (i) all complementary to the first strand of the target sequence and (ii) each

25        complementary to a different portion of the right hybridization target, and the complementary portions of the left set primers can be (i) all complementary to the second strand of the target sequence and (ii) each complementary to a different portion of the left hybridization target.

          The right and left set of primers each can have 3 or more primers. The right and left

30        set of primers each can have 4 or more primers. The right and left set of primers each can have 5 or more primers. The right and left set of primers each can have the same number of primers. The target sequence can be a nucleic acid sample of substantial complexity, and the set of primers can comprise primers having random nucleotide sequences. The target

sequence can be a sample of genomic nucleic acid. The primers can be from 5 to 20 nucleotides in length. The primers can be from 5 to 10 nucleotides in length. The primers can be 6, 7, or 8 nucleotides in length. The primers can be 6 nucleotides in length. The primers can be all of the same length. Each primer can comprise a constant portion and a

5      random portion, where the constant portion of each primer can have the same nucleotide sequence and the random portion of each primer can have a random nucleotide sequence.

The target sequence can be concatenated DNA. The concatenated DNA can be concatenated with linkers. Each linker can comprise a primer complement portion, each primer can comprise a complementary portion, and the complementary portion of each

10     primer can be complementary to the complementary portion of the linkers. The set of primers can comprise primers having random nucleotide sequences. Each primer can comprise a constant portion and a random portion, the constant portion of each primer can have the same nucleotide sequence and the random portion of each primer can have a random nucleotide sequence. The concatenated DNA can be formed by ligating DNA

15     fragments together. The DNA fragments can be cDNA made from mRNA. The mRNA can comprise a mixture of mRNA isolated from cells. The target sequence need not be a nucleic acid molecule made up of multiple tandem repeats of a single sequence that was synthesized by rolling circle replication.

The primers can comprise nucleotides, where one or more of the nucleotides can be

20     ribonucleotides. From about 10% to about 50% of the nucleotides can be ribonucleotides. About 50% or more of the nucleotides can be ribonucleotides. All of the nucleotides can be ribonucleotides. The primers can comprise nucleotides, where one or more of the nucleotides can be 2'-O-methyl ribonucleotides. From about 10% to about 50% of the nucleotides can be 2'-O-methyl ribonucleotides. About 50% or more of the nucleotides can

25     be 2'-O-methyl ribonucleotides. All of the nucleotides can be 2'-O-methyl ribonucleotides. The primers can comprise nucleotides and the nucleotides can be a mixture of ribonucleotides and 2'-O-methyl ribonucleotides. The primers can comprise nucleotides, the nucleotides can comprise bases, and one or more of the bases can be universal bases. At least one of the universal bases can be 3-nitropyrrole. The universal base can be 5-

30     nitroindole. From about 10% to about 50% of the bases can be universal bases. About 50% or more of the bases can be universal bases. All of the bases can be universal bases.

The target sample can be a biopsy sample, a blood sample, a urine sample, a cell sample, or a tissue sample. The target sample is a needle aspiration biopsy sample. Nucleic

acids in the target sample need not be separated from other material in the target sample. The target sample can be a crude cell lysate. The target sample need not be processed beyond cell lysis. The replicated strands can be analyzed. The replicated strands can be analyzed using one or more DNA chips. The replicated strands can be analyzed by

5 hybridization. The replicated strands can be analyzed by nucleic acid sequencing. The replicated strands can be stored prior to, following, or both prior to and following their analysis. The target sample can be a blood sample, a urine sample, a semen sample, a lymphatic fluid sample, a cerebrospinal fluid sample, amniotic fluid sample, a biopsy sample, a needle aspiration biopsy sample, a cancer sample, a tumor sample, a tissue

10 sample, a cell sample, a cell lysate sample, a crude cell lysate sample, a forensic sample, an archeological sample, an infection sample, a nosocomial infection sample, a production sample, a drug preparation sample, a biological molecule production sample, a protein preparation sample, a lipid preparation sample, a carbohydrate preparation sample, or a combination thereof.

15        The target sample can be a blood sample. The target sample can be a needle aspiration biopsy sample. The target sample can be a crude cell lysate sample. The target sample can be a nosocomial infection sample. The sample can be derived from a patient. The target sample can be a biological molecule production sample. Production of replicated strands can indicate the presence of nucleic acids in the sample. The amount of replicated

20 strands produced can indicate the amount of nucleic acids in the sample. The target sample can be a drug preparation sample. The target sample can be a tumor sample. The target sample can be an amniotic fluid sample. The replicated strands produced from the target sample can represent a nucleic acid fingerprint of the sample.

        The second target sample can be a sample from the same type of organism as the

25 first target sample. The second target sample can be a sample from the same type of tissue as the first target sample. The second target sample can be a sample from the same organism as the first target sample. The second target sample can be obtained at a different time than the first target sample. The second target sample can be a sample from a different organism than the first target sample. The second target sample can be a sample from a

30 different type of tissue than the first target sample. The second target sample can be a sample from a different species of organism than the first target sample. The second target sample can be a sample from a different strain of organism than the first target sample. The

227

second target sample can be a sample from a different cellular compartment than the first target sample.

A circular nucleic acid molecule can comprise the target sequence. The circular nucleic acid molecule can be produced by digesting genomic DNA with a restriction endonuclease, and circularizing the digested DNA. The digested DNA can be circularized with DNA or RNA ligase. The digested DNA can be circularized with a splint or adaptor. The target sequence can comprise an amplification target and a hybridization target, the hybridization target can flank the amplification target, the set of primers can comprise a plurality of primers, each primer can comprise a complementary portion, and the complementary portions of the primers each can be complementary to a different portion of the hybridization target.

The set of primers can comprise a right set of primers and a left set of primers, the target sequence can be double-stranded, having a first and a second strand, the hybridization target can comprise a right and left hybridization target, and the right hybridization target can flank the amplification target on one end and the left hybridization target flanks the amplification target on the other end. The complementary portions of the right set primers can be (i) all complementary to the first strand of the target sequence and (ii) each complementary to a different portion of the right hybridization target, and the complementary portions of the left set primers can be (i) all complementary to the second strand of the target sequence and (ii) each complementary to a different portion of the left hybridization target.

The circular nucleic acid molecule can be produced by circularizing cDNA. The circular nucleic acid molecule is produced by circularizing mRNA/cDNA hybrid. The mRNA/cDNA hybrid can be circularized with DNA or RNA ligase. The mRNA/cDNA hybrid can be circularized with a splint or adaptor.

Also disclosed is a method of amplifying a target nucleic acid sequence where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. Nucleic acids in the target sample are not separated from other material in the target sample. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

228

The method can further comprise bringing into contact a set of primers, DNA polymerase, and a second target sample, and incubating the second target sample under conditions that promote replication of the target sequence. The second target sample is not subjected to denaturing conditions. Replication of the target sequence results in replicated

5    strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

The target sample can be a crude cell lysate. The target sample need not be processed beyond cell lysis. The target sample can be a blood sample, a urine sample, a semen sample, a lymphatic fluid sample, a cerebrospinal fluid sample, amniotic fluid

10   sample, a biopsy sample, a needle aspiration biopsy sample, a cancer sample, a tumor sample, a tissue sample, a cell sample, a cell lysate sample, a crude cell lysate sample, a forensic sample, an archeological sample, an infection sample, a nosocomial infection sample, a production sample, a drug preparation sample, a biological molecule production sample, a protein preparation sample, a lipid preparation sample, a carbohydrate preparation

15   sample, or a combination thereof. The target sample can be a blood sample. The target sample can be a needle aspiration biopsy sample. The target sample can be a crude cell lysate sample. The target sample can be a nosocomial infection sample. The sample can be derived from a patient. The target sample can be a biological molecule production sample.

Production of replicated strands can indicate the presence of nucleic acids in the

20   sample. The amount of replicated strands produced can indicate the amount of nucleic acids in the sample. The target sample can be a drug preparation sample. The target sample can be a tumor sample. The target sample can be an amniotic fluid sample. The replicated strands produced from the target sample can represent a nucleic acid fingerprint of the sample.

25   The second target sample can be a sample from the same type of organism as the first target sample. The second target sample can be a sample from the same type of tissue as the first target sample. The second target sample can be a sample from the same organism as the first target sample. The second target sample can be obtained at a different time than the first target sample. The second target sample can be a sample from a different

30   organism than the first target sample. The second target sample can be a sample from a different type of tissue than the first target sample. The second target sample can be a sample from a different species of organism than the first target sample. The second target sample can be a sample from a different strain of organism than the first target sample. The

second target sample can be a sample from a different cellular compartment than the first target sample.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. The target sample is a crude cell lysate. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. The primers are 5, 6, 7, 8, 9, or 10 nucleotides long. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. The primers each contain at least one modified nucleotide such that the primers are nuclease resistant. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. The primer-target sample is not subjected to denaturing conditions, the primers are 6 nucleotides long, the primers each contain at least one modified nucleotides such that the primers are nuclease resistant, and the DNA polymerase is φ29 DNA polymerase. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. Replication of the target sequence results in replicated strands, where

5      during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand. The method further comprises diluting the replicated strands, bringing into contact a set of primers, DNA polymerase, and the diluted replicated strands, and incubating the replicated strands under conditions that promote replication of the target sequence. Replication of the target

10     sequence results in additional replicated strands, where during replication at least one of the additional replicated strands is displaced from the target sequence by strand displacement replication of another additional replicated strand.

The method can further comprise performing the following operation one or more times: diluting the additional replicated strands, bringing into contact a set of primers, DNA

15     polymerase, and the diluted replicated strands, and incubating the replicated strands under conditions that promote replication of the target sequence. Replication of the target sequence results in additional replicated strands, where during replication at least one of the additional replicated strands is displaced from the target sequence by strand displacement replication of another additional replicated strand.

20     Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises,

(a)   mixing a set of primers with a target sample, to produce a primer-target sample mixture, and incubating the primer-target sample mixture under conditions that promote hybridization between the primers and the target sequence in the primer-target sample

25     mixture, where the primer-target sample is not subjected to denaturing conditions, and

(b)   mixing DNA polymerase with the primer-target sample mixture, to produce a polymerase-target sample mixture, and incubating the polymerase-target sample mixture under conditions that promote replication of the target sequence.

The set of primers comprises a right set of primers and a left set of primers, the

30     target sequence is double-stranded, having a first and a second strand, where the right set primers are all complementary to the first strand of the target sequence and the left set primers are all complementary to the second strand of the target sequence. Replication of the target sequence results in replicated strands, where during replication at least one of the

231

replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand. The right set of primers can have 4 or more primers and the left set of primers has 4 or more primers.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises,

(a)   mixing a set of primers with a target sample, to produce a primer-target sample mixture, and incubating the primer-target sample mixture under conditions that promote hybridization between the primers and the target sequence in the primer-target sample mixture, where the primer-target sample is not subjected to denaturing conditions, and

(b)   mixing DNA polymerase with the primer-target sample mixture, to produce a polymerase-target sample mixture, and incubating the polymerase-target sample mixture under conditions that promote replication of the target sequence.

Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand. The target sequence is a nucleic acid sample of substantial complexity, and the set of primers comprises primers having random nucleotide sequences.

Also disclosed is a method of amplifying a target nucleic acid sequence, the method comprising,

(a)   mixing a set of primers with a target sample, to produce a primer-target sample mixture, and incubating the primer-target sample mixture under conditions that promote hybridization between the primers and the target sequence in the primer-target sample mixture, where the primer-target sample is not subjected to denaturing conditions, and

(b)   mixing DNA polymerase with the primer-target sample mixture, to produce a polymerase-target sample mixture, and incubating the polymerase-target sample mixture under conditions that promote replication of the target sequence.

All of the primers in the set of primers are complementary to the same strand in the target sequence. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand. The set of primers can have 3 or more primers.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises,

(a) mixing a set of primers with a target sample, to produce a primer-target sample mixture, and incubating the primer-target sample mixture under conditions that promote hybridization between the primers and the target sequence in the primer-target sample mixture, where the primer-target sample is not subjected to denaturing conditions, and

5          (b) mixing DNA polymerase with the primer-target sample mixture, to produce a polymerase-target sample mixture, and incubating the polymerase-target sample mixture under conditions that promote replication of the target sequence.

Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by

10     strand displacement replication of another replicated strand. The target sequence is a nucleic acid sample of substantial complexity, and the set of primers comprises primers having random nucleotide sequences. Each primer comprises a constant portion and a random portion, where the constant portion of each primer has the same nucleotide sequence and the random portion of each primer has a random nucleotide sequence.

15          Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises,

(a) mixing a set of primers with a target sample, to produce a primer-target sample mixture, and incubating the primer-target sample mixture under conditions that promote hybridization between the primers and the target sequence in the primer-target sample

20     mixture, where the primer-target sample is not subjected to denaturing conditions, and

(b) mixing DNA polymerase with the primer-target sample mixture, to produce a polymerase-target sample mixture, and incubating the polymerase-target sample mixture under conditions that promote replication of the target sequence.

Replication of the target sequence results in replicated strands, where during

25     replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand. The conditions that promote replication of the target sequence do not involve thermal cycling and the target sequence is concatenated DNA.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the

30     method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. The target sample is not subjected to denaturing conditions. Replication of the target sequence results in replicated strands, where during replication at least one of the

replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand. The set of primers can have 3 or more primers complementary to the same strand of the target sequence.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. The target sample is not subjected to denaturing conditions. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand. The target sequence is a nucleic acid sample of substantial complexity, and the set of primers comprises primers having random nucleotide sequences.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. The target sample is not subjected to denaturing conditions. All of the primers in the set of primers are complementary to the same strand in the target sequence. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand. The set of primers can have 3 or more primers.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. The target sample is not subjected to denaturing conditions. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand. The target sequence is a nucleic acid sample of substantial complexity, and the set of primers comprises primers having random nucleotide sequences. Each primer comprises a constant portion and a random portion, where the constant portion of each primer has the same nucleotide sequence and the random portion of each primer has a random nucleotide sequence.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target

sample, and incubating the target sample under conditions that promote replication of the target sequence. The target sample is not subjected to denaturing conditions. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication

5       of another replicated strand. The conditions that promote replication of the target sequence do not involve thermal cycling, and the target sequence is concatenated DNA.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the

10      target sequence. The target sample is not subjected to heat denaturing conditions. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Also disclosed is a method of labeling nucleic acids produced by strand

15      displacement replication, where the method comprises, labeling nucleic acids produced by strand displacement replication using terminal deoxynucleotidyl transferase. The replicated strands can be labeled by the addition of modified nucleotides to the 3' ends of the nucleic acids. The modified nucleotides can be biotinylated nucleotides, fluorescent nucleotides, 5 methyl dCTP, BrdUTP, or 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphates.

20      Also disclosed is a method of labeling nucleic acids produced by strand displacement replication, where the method comprises, incorporating modified nucleotides into nucleic acids produced by strand displacement replication during replication.

Also disclosed is a method of attaching nucleic acids produced by strand displacement replication, where the method comprises, adding modified nucleotides to the

25      3' ends of nucleic acids produced by strand displacement replication using terminal deoxynucleotidyl transferase, and reacting the modified nucleotides with a solid-state support thereby attaching the nucleic acids to the solid-state support.

Also disclosed is a microarray comprising nucleic acids produced by strand displacement replication coupled or adhered to a solid-state substrate.

30      Also disclosed is a method of generating probes based on a target nucleic acid sequence, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. Replication of the target sequence results in

replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand. The replicated strands are used as hybridization probes. The method can further comprise labeling the hybridization probes using terminal deoxynucleotidyl transferase.

5      Also disclosed is a method of amplifying messenger RNA, where the method comprises, reverse transcribing messenger RNA to produce a first strand cDNA, bringing into contact a set of primers, DNA polymerase, and the first strand cDNA, and incubating under conditions that promote replication of the first strand cDNA. Replication of the first strand cDNA results in replicated strands, where during replication at least one of the

10     replicated strands is displaced from the first strand cDNA by strand displacement replication of another replicated strand. The method can further comprise, prior to replication, degrading the messenger RNA using RNAse H.

The method can further comprise bringing into contact a set of primers, DNA polymerase, and a second target sample, and incubating the second target sample under

15     conditions that promote replication of the target sequence. The second target sample is not subjected to denaturing conditions. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the

20     method comprises, partially degrading RNA in a target sample, bringing into contact DNA polymerase, and the target sample, and incubating the target sample under conditions that promote replication of the target sequence. Replication of the target sequence results in replicated strands, where during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

25     Also disclosed is a method of comparative genome hybridization, where the method comprises, hybridizing nucleic acids produced by strand displacement replication of a first sample with nucleic acids produced by strand displacement replication of a second sample. Hybridization can be carried out in the absence of CotI DNA.

Also disclosed is a method of amplifying a target nucleic acid sequence, where the

30     method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, and incubating the target sample under conditions that promote replication of the target sequence. A circular nucleic acid molecule comprises the target sequence. Replication of the target sequence results in replicated strands, where during replication at

least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

The set of primers can comprise primers having random nucleotide sequences. The primers can be 5, 6, 7, or 8 nucleotides long. The primers can be 6, 7, or 8 nucleotides long. The primers can be 6 nucleotides long. The modified nucleotides can be biotinylated nucleotides, fluorescent nucleotides, 5 methyl dCTP, BrdUTP, or 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphates. The modified nucleotides can be 5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphates and the replicated strands can be labeled by reacting labels with the incorporated 5-(3-aminoallyl)-2'-deoxyuridines. The labels can be fluorescein isothiocyanate, 5,6-carboxymethyl fluorescein, Texas red, nitrobenz-2-oxa-1,3-diazol-4-yl, coumarin, dansyl chloride, rhodamine, amino-methyl coumarin, Eosin, Erythrosin, BODIPY®, Cascade Blue®, Oregon Green®, pyrene, lissamine, xanthene, acridine, oxazines, phycoerythrin, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, or a combination thereof.

The replicated strands can be used as elements in a microarray. The replicated strands can be cleaved prior to use as hybridization probes. The replicated strands can be cleaved with DNAse I. The hybridization probes can be labeled by the addition of modified nucleotides to the 3' ends of the replicated strands. The modified nucleotides can be reacted with a solid-state support thereby attaching the replicated strands to the solid-state support.

The target sample can be a blood sample, a urine sample, a semen sample, a lymphatic fluid sample, a cerebrospinal fluid sample, amniotic fluid sample, a biopsy sample, a needle aspiration biopsy sample, a cancer sample, a tumor sample, a tissue sample, a cell sample, a cell lysate sample, a crude cell lysate sample, a forensic sample, an archeological sample, an infection sample, a nosocomial infection sample, a production sample, a drug preparation sample, a biological molecule production sample, a protein preparation sample, a lipid preparation sample, a carbohydrate preparation sample, or a combination thereof. The target sample can be a blood sample. The target sample is a needle aspiration biopsy sample. The target sample can be a crude cell lysate sample. The target sample can be a nosocomial infection sample. The sample can contain both human and non-human nucleic acids. The non-human nucleic acid can be amplified preferentially by the use of primers specific for the non-human nucleic acid. The human nucleic acid can be amplified preferentially by the use of primers specific for human nucleic acid. The sample can be derived from a patient. The target sample can be a biological molecule production sample. Production of replicated strands can indicate the presence of nucleic

acids in the sample. The amount of replicated strands produced can indicate the amount of nucleic acids in the sample. The target sample can be a drug preparation sample. The target sample can be a tumor sample. The target sample can be an amniotic fluid sample. The replicated strands produced from the target sample can represent a nucleic acid fingerprint

5     of the sample.

The second target sample can be a sample from the same type of organism as the first target sample. The second target sample can be a sample from the same type of tissue as the first target sample. The second target sample can be a sample from the same organism as the first target sample. The second target sample can be obtained at a different

10     time than the first target sample. The second target sample can be a sample from a different organism than the first target sample. The second target sample can be a sample from a different type of tissue than the first target sample. The second target sample can be a sample from a different species of organism than the first target sample. The second target sample can be a sample from a different strain of organism than the first target sample. The

15     second target sample can be a sample from a different cellular compartment than the first target sample.

The circular nucleic acid molecule can be produced by digesting genomic DNA with a restriction endonuclease, and circularizing the digested DNA. The digested DNA can be circularized with DNA or RNA ligase. The digested DNA can be circularized with a splint

20     or adaptor. The target sequence can comprises an amplification target and a hybridization target, the hybridization target can flank the amplification target, the set of primers can comprise a plurality of primers, each primer can comprise a complementary portion, and the complementary portions of the primers each can be complementary to a different portion of the hybridization target.

25     The set of primers can comprise a right set of primers and a left set of primers, the target sequence can be double-stranded, having a first and a second strand, the hybridization target can comprise a right and left hybridization target, the right hybridization target can flank the amplification target on one end and the left hybridization target can flank the amplification target on the other end. The complementary portions of the right set primers

30     can be (i) all complementary to the first strand of the target sequence and (ii) each complementary to a different portion of the right hybridization target, and the complementary portions of the left set primers can be (i) all complementary to the second

strand of the target sequence and (ii) each complementary to a different portion of the left hybridization target.

The circular nucleic acid molecule can be produced by circularizing cDNA. The circular nucleic acid molecule can be produced by circularizing mRNA/cDNA hybrid. The mRNA/cDNA hybrid can be circularized with DNA or RNA ligase. The mRNA/cDNA hybrid can be circularized with a splint or adaptor.

Also disclosed is a method of amplifying a whole genome, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, where the target sample comprises a whole genome, and incubating the target sample under conditions that promote replication of the genome. The target sample is not subjected to denaturing conditions. Replication of the genome results in replicated strands, where during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand. The primers can be 6 nucleotides long, the primers each can contain at least one modified nucleotide such that the primers are nuclease resistant, and the DNA polymerase can be φ29 DNA polymerase. The primers can be of random nucleotide composition, the primers each can contain at least one modified nucleotide such that the primers are nuclease resistant, and the DNA polymerase can be φ29 DNA polymerase.

Also disclosed is a method of amplifying a chromosome, where the method comprises, bringing into contact a set of primers, DNA polymerase, and a target sample, where the target sample comprises a chromosome, and incubating the target sample under conditions that promote replication of the chromosome, where the target sample is not subjected to denaturing conditions. Replication of the chromosome results in replicated strands, where during replication at least one of the replicated strands is displaced from the chromosome by strand displacement replication of another replicated strand. The primers can be 6 nucleotides long, the primers each can contain at least one modified nucleotide such that the primers are nuclease resistant, and the DNA polymerase can be φ29 DNA polymerase. The primers can be of random nucleotide composition, the primers each can contain at least one modified nucleotide such that the primers are nuclease resistant, and the DNA polymerase can be φ29 DNA polymerase.

Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome, neutralizing the cell lysate to form a neutralized cell lysate, and incubating the neutralized

239

cell lysate under conditions that promote replication of the genome, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

5        Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome, neutralizing the cell lysate, wherein the cell lysate is not purified, and incubating the cell lysate under conditions that promote replication of the genome, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated

10     strands is displaced from the genome by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome, neutralizing the cell lysate, wherein nucleic acids in the cell lysate are not separated from

15     other material in the cell lysate, and incubating the cell lysate under conditions that promote replication of the genome, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Furthermore, disclosed are methods of amplifying a whole genome, the methods

20     comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome, neutralizing the cell lysate to form a neutralized cell lysate, and incubating the neutralized cell lysate under conditions that promote replication of the genome, wherein the neutralized cell lysate is not subjected to denaturing conditions, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated

25     strands is displaced from the genome by strand displacement replication of another replicated strand.

Also disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome, neutralizing the cell lysate to form a neutralized cell lysate, and incubating the neutralized

30     cell lysate under conditions that promote replication of the genome, wherein the neutralized cell lysate is subjected to heat denaturing conditions, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands

is displaced from the genome by strand displacement replication of another replicated strand.

Methods of amplifying a whole genome are also disclosed, the methods comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome,

5    neutralizing the cell lysate to form a neutralized cell lysate, wherein nucleic acids in the neutralized cell lysate are not separated from other material in the cell lysate, and incubating the neutralized cell lysate under conditions that promote replication of the genome, wherein the neutralized cell lysate is not subjected to denaturing conditions, wherein replication of the genome results in replicated strands, wherein during replication at least one of the

10    replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a neutralized cell lysate, and bringing into contact a set of primers, DNA polymerase, and the neutralized cell lysate,

15    wherein the neutralized cell lysate comprises a whole genome, and incubating the neutralized cell lysate under conditions that promote replication of the genome, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

20    Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate, bringing into contact a set of primers, DNA polymerase, and the cell lysate, wherein the cell lysate comprises a whole genome, wherein the cell lysate is not purified, and incubating the cell lysate under conditions that promote replication of the genome, wherein replication of the genome

25    results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate, bringing into contact a set of

30    primers, DNA polymerase, and the cell lysate, wherein the cell lysate comprises a whole genome, wherein nucleic acids in the cell lysate are not separated from other material in the cell lysate, and incubating the cell lysate under conditions that promote replication of the genome, wherein replication of the genome results in replicated strands, wherein during

replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a neutralized cell lysate, and bringing into contact a set of primers, DNA polymerase, and the neutralized cell lysate, wherein the neutralized cell lysate comprises a whole genome, and incubating the neutralized cell lysate under conditions that promote replication of the genome, wherein the neutralized cell lysate is not subjected to denaturing conditions, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Also disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a neutralized cell lysate, and bringing into contact a set of primers, DNA polymerase, and the neutralized cell lysate, wherein the neutralized cell lysate comprises a whole genome, and incubating the neutralized cell lysate under conditions that promote replication of the genome, wherein the neutralized cell lysate is subjected to heat denaturing conditions, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a whole genome, the method comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a neutralized cell lysate, and bringing into contact a set of primers, DNA polymerase, and the neutralized cell lysate, wherein the neutralized cell lysate comprises a whole genome, wherein nucleic acids in the neutralized cell lysate are not separated from other material in the neutralized cell lysate, and incubating the neutralized cell lysate under conditions that promote replication of the genome, wherein the neutralized cell lysate is not subjected to denaturing conditions, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Also disclosed are methods of amplifying a target nucleic acid sequence, the method comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a neutralized cell lysate, and incubating the neutralized cell lysate in the presence of a set of

primers and DNA polymerase and under conditions that promote replication of a target
sequence, wherein replication of the neutralized cell lysate results in replicated strands,
wherein during replication at least one of the replicated strands is displaced from the target
sequence by strand displacement replication of another replicated strand.

5          Disclosed are methods of amplifying a target nucleic acid sequence, the methods
comprising, lysing cells to form a cell lysate, neutralizing the cell lysate, and incubating the
cell lysate in the presence of a set of primers and DNA polymerase and under conditions
that promote replication of a target sequence, wherein the cell lysate is not purified, wherein
replication of the cell lysate results in replicated strands, wherein during replication at least
10        one of the replicated strands is displaced from the target sequence by strand displacement
replication of another replicated strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the methods
comprising, lysing cells to form a cell lysate, neutralizing the cell lysate, and incubating the
cell lysate in the presence of a set of primers and DNA polymerase and under conditions
15        that promote replication of a target sequence, wherein nucleic acids in the cell lysate are not
separated from other material in the cell lysate, wherein replication of the cell lysate results
in replicated strands, wherein during replication at least one of the replicated strands is
displaced from the target sequence by strand displacement replication of another replicated
strand.

20          Also disclosed are methods of amplifying a target nucleic acid sequence, the
methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a
neutralized cell lysate, and incubating the neutralized cell lysate in the presence of a set of
primers and DNA polymerase and under conditions that promote replication of a target
sequence, wherein the neutralized cell lysate is not subjected to denaturing conditions,
25        wherein replication of the neutralized cell lysate results in replicated strands, wherein during
replication at least one of the replicated strands is displaced from the target sequence by
strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the methods
comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a
30        neutralized cell lysate, and incubating the neutralized cell lysate in the presence of a set of
primers and DNA polymerase and under conditions that promote replication of a target
sequence, wherein the neutralized cell lysate is subjected to denaturing conditions, wherein
replication of the neutralized cell lysate results in replicated strands, wherein during

243

replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a neutralized cell lysate, and incubating the neutralized cell lysate in the presence of a set of primers and DNA polymerase and under conditions that promote replication of a target sequence, wherein nucleic acids in the neutralized cell lysate are not separated from other material in the neutralized cell lysate, wherein the neutralized cell lysate is not subjected to denaturing conditions, wherein replication of the neutralized cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Also disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a neutralized cell lysate, and bringing into contact a set of primers, DNA polymerase, and the neutralized cell lysate, and incubating the neutralized cell lysate under conditions that promote replication of a target sequence, wherein replication of the neutralized cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate, bringing into contact a set of primers, DNA polymerase, and the cell lysate, and incubating the cell lysate under conditions that promote replication of a target sequence, wherein the cell lysate is not purified, wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Also disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate, bringing into contact a set of primers, DNA polymerase, and the cell lysate, and incubating the cell lysate under conditions that promote replication of a target sequence, wherein nucleic acids in the cell lysate are not separated from other material in the cell lysate, wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the

244

replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a

5 neutralized cell lysate, and bringing into contact a set of primers, DNA polymerase, and the neutralized cell lysate, and incubating the neutralized cell lysate under conditions that promote replication of a target sequence, wherein the neutralized cell lysate is not subjected to denaturing conditions, wherein replication of the neutralized cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is

10 displaced from the target sequence by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a neutralized cell lysate, and bringing into contact a set of primers, DNA polymerase, and the

15 neutralized cell lysate, and incubating the neutralized cell lysate under conditions that promote replication of a target sequence, wherein the neutralized cell lysate is subjected to heat denaturing conditions, wherein replication of the neutralized cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated

20 strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the method comprising, lysing cells to form a cell lysate, neutralizing the cell lysate to form a neutralized cell lysate, and bringing into contact a set of primers, DNA polymerase, and the neutralized cell lysate, and incubating the neutralized cell lysate under conditions that

25 promote replication of a target sequence, wherein nucleic acids in the neutralized cell lysate are not separated from other material in the neutralized cell lysate, wherein the neutralized cell lysate is not subjected to denaturing conditions, wherein replication of the neutralized cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication

30 of another replicated strand.

Disclosed are methods of amplifying a whole genome, the method comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome, and incubating the cell lysate under conditions that promote replication of the genome, wherein

245

replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Also disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome, wherein the cell lysate is not purified, and incubating the cell lysate under conditions that promote replication of the genome, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome, wherein nucleic acids in the cell lysate are not separated from other material in the cell lysate, and incubating the cell lysate under conditions that promote replication of the genome, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome, and incubating the cell lysate under conditions that promote replication of the genome, wherein the cell lysate is not subjected to denaturing conditions, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Also disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome, and incubating the cell lysate under conditions that promote replication of the genome, wherein the cell lysate is subjected to heat denaturing conditions, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a whole genome, the method comprising, lysing cells to form a cell lysate, wherein the cell lysate comprises a whole genome, wherein nucleic acids in the cell lysate are not separated from other material in the cell lysate, and

246

incubating the cell lysate under conditions that promote replication of the genome, wherein the cell lysate is not subjected to denaturing conditions, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated

5      strand.

Also disclosed are methods of amplifying a whole genome, the method comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA polymerase, and the cell lysate, wherein the cell lysate comprises a whole genome, and incubating the cell lysate under conditions that promote replication of the genome, wherein replication of

10     the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA polymerase,

15     and the cell lysate, wherein the cell lysate comprises a whole genome, wherein the cell lysate is not purified, and incubating the cell lysate under conditions that promote replication of the genome, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

20     Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA polymerase, and the cell lysate, wherein the cell lysate comprises a whole genome, wherein nucleic acids in the cell lysate are not separated from other material in the cell lysate, and incubating the cell lysate under conditions that promote replication of the genome, wherein replication of

25     the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Also disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA polymerase,

30     and the cell lysate, wherein the cell lysate comprises a whole genome, and incubating the cell lysate under conditions that promote replication of the genome, wherein the cell lysate is not subjected to denaturing conditions, wherein replication of the genome results in

247

replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA polymerase,

5      and the cell lysate, wherein the cell lysate comprises a whole genome, and incubating the cell lysate under conditions that promote replication of the genome, wherein the cell lysate is subjected to heat denaturing conditions, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

10     Disclosed are methods of amplifying a whole genome, the methods comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA polymerase, and the cell lysate, wherein the cell lysate comprises a whole genome, wherein nucleic acids in the cell lysate are not separated from other material in the cell lysate, and incubating the cell lysate under conditions that promote replication of the genome, wherein the cell lysate

15     is not subjected to denaturing conditions, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Also disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, and incubating the cell lysate in the

20     presence of a set of primers and DNA polymerase and under conditions that promote replication of a target sequence, wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the methods

25     comprising, lysing cells to form a cell lysate, and incubating the cell lysate in the presence of a set of primers and DNA polymerase and under conditions that promote replication of a target sequence, wherein the cell lysate is not purified, wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another

30     replicated strand.

Also disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, and incubating the cell lysate in the presence of a set of primers and DNA polymerase and under conditions that promote

replication of a target sequence, wherein nucleic acids in the cell lysate are not separated from other material in the cell lysate, wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated

5    strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, and incubating the cell lysate in the presence of a set of primers and DNA polymerase and under conditions that promote replication of a target sequence, wherein the cell lysate is not subjected to denaturing conditions, wherein

10   replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Also disclosed are methods of amplifying a target nucleic acid sequence, the method comprising, lysing cells to form a cell lysate, and incubating the cell lysate in the presence

15   of a set of primers and DNA polymerase and under conditions that promote replication of a target sequence, wherein the cell lysate is subjected to heat denaturing conditions, wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

20   Disclosed are methods of amplifying a target nucleic acid sequence, the method comprising, lysing cells to form a cell lysate, and incubating the cell lysate in the presence of a set of primers and DNA polymerase and under conditions that promote replication of a target sequence, wherein nucleic acids in the cell lysate are not separated from other material in the cell lysate, wherein the cell lysate is not subjected to denaturing conditions,

25   wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the method comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA

30   polymerase, and the cell lysate, and incubating the cell lysate under conditions that promote replication of a target sequence, wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA polymerase, and the cell lysate, and incubating the cell lysate under conditions that promote replication of a target sequence, wherein the cell lysate is not purified, wherein replication

5    of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA

10   polymerase, and the cell lysate, and incubating the cell lysate under conditions that promote replication of a target sequence, wherein nucleic acids in the cell lysate are not separated from other material in the cell lysate, wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated

15   strand.

Also disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA polymerase, and the cell lysate, and incubating the cell lysate under conditions that promote replication of a target sequence, wherein the cell lysate is not subjected to

20   denaturing conditions, wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

Disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA

25   polymerase, and the cell lysate, and incubating the cell lysate under conditions that promote replication of a target sequence, wherein the cell lysate is subjected to heat denaturing conditions, wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

30   Disclosed are methods of amplifying a target nucleic acid sequence, the methods comprising, lysing cells to form a cell lysate, bringing into contact a set of primers, DNA polymerase, and the cell lysate, and incubating the cell lysate under conditions that promote replication of a target sequence, wherein nucleic acids in the cell lysate are not separated

250

from other material in the cell lysate, wherein the cell lysate is not subjected to denaturing conditions, wherein replication of the cell lysate results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the target sequence by strand displacement replication of another replicated strand.

5          Any of the disclosed methods can be performed wherein the neutralized cell lysate is not subjected to denaturing conditions or wherein the neutralized cell lysate is subjected to heat denaturing conditions.

Disclosed are kits for amplifying a whole genome, the kits comprising a solution for lysis, a solution for neutralization, a set of primers, and a DNA polymerase.

10         Also disclosed are kits for amplifying a whole genome, the kits comprising a solution for cell lysis, a solution for neutralization of a cell lysate, a set of primers, and a DNA polymerase.

Disclosed are kits for amplifying a whole genome, the kits comprising a solution for lysing cells, a solution for neutralizing lysed cells, a set of primers, and a DNA polymerase.

15         Also disclosed are kits for amplifying a whole genome, the kits comprising a composition for lysis, a composition for neutralization, a set of primers, and a DNA polymerase.

Disclosed are kits for amplifying a whole genome, the kits comprising a composition for cell lysis, a composition for neutralization of a cell lysate, a set of primers, 20 and a DNA polymerase.

Disclosed are kits for amplifying a whole genome, the kits comprising a composition for lysing cells, a composition for neutralizing lysed cells, a set of primers, and a DNA polymerase.

Disclosed are kits for amplifying a whole genome, the kits comprising a solution for 25 lysis, wherein the solution for lysis is alkaline, a solution for neutralization, a set of primers, wherein the primers are 6 nucleotides long, wherein the primers each contain at least one modified nucleotide such that the primers are nuclease resistant, and a DNA polymerase, wherein the DNA polymerase is φ29 DNA polymerase.

Disclosed are kits for amplifying a whole genome, the kits comprising a solution for 30 cell lysis, wherein the solution for cell lysis is alkaline, a solution for neutralization of a cell lysate, a set of primers, wherein the primers are 6 nucleotides long, wherein the primers each contain at least one modified nucleotide such that the primers are nuclease resistant, and a DNA polymerase, wherein the DNA polymerase is φ29 DNA polymerase.

Also disclosed are kits for amplifying a whole genome, the kits comprising a solution for lysing cells, wherein the solution for lysing cells is alkaline, a solution for neutralizing lysed cells, a set of primers, wherein the primers are 6 nucleotides long, wherein the primers each contain at least one modified nucleotide such that the primers are nuclease resistant, and a DNA polymerase, wherein the DNA polymerase is $\phi29$ DNA polymerase.

Disclosed are methods of amplifying a whole genome, the method comprising, exposing cells to alkaline conditions to form a cell lysate, wherein the cell lysate comprises a whole genome, reducing the pH of the cell lysate to form a stabilized cell lysate, and incubating the stabilized cell lysate under conditions that promote replication of the genome, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand displacement replication of another replicated strand.

Also disclosed are methods, wherein the cells are exposed to alkaline conditions by mixing the cells with a lysis solution or wherein the lysis solution comprises a base or wherein the base is an aqueous base.

Also disclosed are methods, wherein the base is potassium hydroxide, sodium hydroxide, potassium acetate, sodium acetate, ammonium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, calcium carbonate, ammonia, aniline, benzylamine, n-butylamine, diethylamine, dimethylamine, diphenylamine, ethylamine, ethylenediamine, methylamine, N-methylaniline, morpholine, pyridine, triethylamine, trimethylamine, aluminum hydroxide, rubidium hydroxide, cesium hydroxide, strontium hydroxide, barium hydroxide, or DBU (1,8-diazobicyclo[5,4,0]undec-7-ene).

Disclosed are methods, wherein the base is potassium hydroxide.

Also disclosed are methods, wherein the lysis solution comprises 400 mM KOH and/or wherein the lysis solution comprises 100 mM dithiothreitol, and 10 mM EDTA or wherein the lysis solution consists of 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA.

Disclosed are methods, wherein the lysis solution comprises a plurality of basic agents and/or wherein the cells are mixed with an equal volume of the lysis solution.

Disclosed are methods, wherein the lysis solution comprises a buffer, as well as methods wherein the buffer is a phosphate buffer, Good buffer, BES, BICINE, CAPS,

EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES, TRICINE, sodium cacodylate, sodium citrate, triethylammonium acetate, triethylammonium bicarbonate, Tris, Bis-tris, or Bis-tris propane. Also disclosed are methods, wherein the buffer is Tris-HCl at pH 4.1. The disclosed methods can comprises a plurality of buffering agents.

5          Also disclosed are methods, wherein the pH of the cell lysate is reduced to the range of about pH 7.0 to about pH 6.8.

Also disclosed are methods, wherein the pH of the cell lysate is reduced by mixing the cell lysate with a stabilization solution. Disclosed are methods wherein the stabilization solution comprises 800 mM Tris-HCl, pH 4.1 as well as methods, wherein the stabilization

10        solution comprises a plurality of buffering agents and/or wherein the cell lysate is mixed with an equal volume of the stabilization solution.

Disclosed are methods, wherein the stabilization solution comprises an acid as well as methods wherein the acid is hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid, acetylsalicylic acid, ascorbic acid, carbonic acid, citric acid, formic acid, nitric acid,

15        perchloric acid, HF, HBr, HI, $H_2S$, HCN, HSCN, HClO, monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, or a carboxylic acid. Disclosed are methods wherein the carboxylic acid is ethanoic, propanoic, or butanoic acid. Disclosed are methods, wherein the stabilization solution comprises a plurality of acidic agents.

Disclosed are methods, wherein the pH of the cell lysate is reduced to about pH 9.0

20        or below, about pH 8.5 or below about pH 8.0 or below, or about pH 7.5 or below.

Also disclosed are methods, wherein the pH of the cell lysate is reduced to the range of about pH 9.0 to about pH 6.0, about pH 9.0 to about pH 7.0, about pH 9.0 to about pH 7.5, pH 9.0 to about pH 8.0, pH 8.5 to about pH 6.0, pH 8.5 to about pH 7.0, pH 8.5 to about pH 7.5, pH 8.5 to about pH 8.0, pH 8.0 to about pH 6.0, pH 8.0 to about pH 6.5, pH

25        8.0 to about pH 7.0, pH 8.0 to about pH 7.5, pH 7.5 to about pH 6.0, or pH 7.5 to about pH 7.0.

Disclosed are methods, wherein nucleic acids in the cell lysate and the stabilized cell lysate are not separated from other material in the cell lysate, wherein the cell lysate and the stabilized cell lysate are not subjected to purification prior to the incubation, wherein the

30        purification comprises separation of nucleic acids in the cell lysate from other material in the cell lysate, wherein the purification comprises centrifugation, extraction, chromatography, filtration, dialysis, or a combination of these, wherein the purification comprises precipitation other than precipitation caused by the alkaline conditions or by the

reduction of the pH, wherein the purification comprises centrifugation, phenol-chloroform extraction, column chromatography, or a combination of these, wherein the cell lysate, stabilized cell lysate, or both are subjected to partial purification prior to the incubation, wherein the partial purification comprises centrifugation, extraction, chromatography,

5      precipitation, filtration, dialysis, or a combination of these, wherein the partial purification comprises centrifugation, phenol-chloroform extraction, column chromatography, or a combination of these, wherein the cell lysate and the stabilized cell lysate are not subjected to substantial purification prior to the incubation, wherein the substantial purification does not include centrifugation, extraction, chromatography, precipitation, filtration, or dialysis,

10     wherein the substantial purification does not include centrifugation, phenol-chloroform extraction, or column chromatography, wherein the cell lysate, stabilized cell lysate, or both are subjected to centrifugation, extraction, chromatography, precipitation, filtration, or dialysis prior to the incubation, wherein the cell lysate, stabilized cell lysate, or both are subjected to centrifugation, phenol-chloroform extraction, or column chromatography prior

15     to the incubation, wherein the substantial purification comprises centrifugation, extraction, chromatography, filtration, dialysis, or a combination of these, wherein the substantial purification comprises precipitation other than precipitation caused by the alkaline conditions or by the reduction of the pH, wherein the substantial purification comprises centrifugation, phenol-chloroform extraction, column chromatography, or a combination of

20     these, wherein the cell lysate and the stabilized cell lysate are not purified prior to the incubation, wherein the cell lysate, stabilized cell lysate, or both are partially purified prior to the incubation, wherein the incubation is substantially isothermic, wherein neither the cell lysate nor the stabilized cell lysate is heated substantially above the temperature of the incubation, wherein neither the cell lysate nor the stabilized cell lysate is subjected to

25     substantial heating above the temperature of the incubation, wherein the cells are not heated substantially above the temperature of the incubation, wherein the cells are not subjected to substantial heating above the temperature of the incubation, wherein the cells are not heated substantially above the temperature at which the cells grow, wherein the cells are not subjected to substantial heating above the temperature at which the cells grow, wherein the

30     cell lysate, stabilized cell lysate, and the cells are not heated substantially above the temperature of the incubation, wherein the cell lysate, stabilized cell lysate, and the cells are not subjected to substantial heating above the temperature of the incubation, wherein the cell lysate, stabilized cell lysate, and the cells are not heated, prior to or during the

incubation, substantially above the temperature at which the cells grow, wherein the cell lysate, stabilized cell lysate, and the cells are not subjected to, prior to or during the incubation, substantial heating above the temperature at which the cells grow prior, wherein neither the cell lysate nor the stabilized cell lysate is heated above a temperature and for a

5      time that would cause notable denaturation of the genome, wherein neither the cell lysate nor the stabilized cell lysate is subjected to heating above a temperature and for a time that would cause notable denaturation of the genome, wherein the cells are not lysed by heat, wherein the cells are not heated above a temperature and for a time that would cause substantial cell lysis in the absence of the alkaline conditions, and/or wherein the cells are

10     not subjected to heating above a temperature and for a time that would cause substantial cell lysis in the absence of the alkaline conditions.

Disclosed are methods of amplifying a whole genome, the methods comprising, exposing cells to alkaline conditions to form a cell lysate, wherein the cell lysate comprises a whole genome, wherein the cells are exposed to alkaline conditions by mixing the cells

15     with a lysis solution, reducing the pH of the cell lysate to form a stabilized cell lysate, wherein the pH of the cell lysate is reduced by mixing the cell lysate with a stabilization solution, and incubating the stabilized cell lysate under conditions that promote replication of the genome, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by

20     strand displacement replication of another replicated strand

Disclosed are methods, wherein the lysis solution comprises potassium hydroxide, such as 400 mM KOH.

Also disclosed are methods wherein the lysis solution comprises 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA or wherein the lysis solution consists of 400 mM

25     KOH, 100 mM dithiothreitol, and 10 mM EDTA.

Disclosed are methods, wherein the cells are mixed with an equal volume of the lysis solution and/or an equal volume of the stabilization solution

Disclosed are methods, wherein the stabilization solution comprises Tris-HCl at pH 4.1 and wherein the stabilization solution comprises 800 mM Tris-HCl, pH 4.1.

30     Also disclosed are methods wherein the stabilization solution consists of 800 mM Tris-HCl, pH 4.1.

Disclosed are methods, wherein the lysis solution consists of 400 mM KOH and 10 mM EDTA, wherein the stabilization solution consists of 800 mM Tris-HCl, pH 4, wherein

the stabilized cell lysate is incubated in the presence of 37.5 mM Tris-HCl, 50 mM KCl, 10 mM MgCl$_2$, 5 mM (NH$_4$)$_2$SO$_4$, 1 mM deoxynucleotide triphosphates, 50 $\mu$M primers, and φ29 DNA Polymerase.

Also disclosed are methods, wherein the stabilized cell lysate is incubated in the

5      presence of 37.5 mM Tris-HCl, 50 mM KCl, 10 mM MgCl$_2$, 5 mM (NH$_4$)$_2$SO$_4$, 1 mM deoxynucleotide triphosphates, 50 $\mu$M primers, and φ29 DNA Polymerase by mixing the stabilized cell lysate with one quarter volume of reaction mix, and φ29 DNA Polymerase, wherein the reaction mix consists of 150 mM Tris-HCl, 200 mM KCl, 40 mM MgCl$_2$, 20 mM (NH$_4$)$_2$SO$_4$, 4 mM deoxynucleotide triphosphates, and 0.2 mM primers.

10     Disclosed are methods of amplifying a whole genome, the methods comprising, exposing cells to alkaline conditions to form a cell lysate, wherein the cell lysate comprises a whole genome, wherein the cells are exposed to alkaline conditions by mixing the cells with a lysis solution, wherein the lysis solution comprises 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA, reducing the pH of the cell lysate to form a stabilized cell

15     lysate, wherein the pH of the cell lysate is reduced by mixing the cell lysate with a stabilization solution, wherein the stabilization solution comprises 800 mM Tris-HCl, pH 4.1, and incubating the stabilized cell lysate under conditions that promote replication of the genome, wherein replication of the genome results in replicated strands, wherein during replication at least one of the replicated strands is displaced from the genome by strand

20     displacement replication of another replicated strand.

Disclosed are methods, wherein the cells are mixed with an equal volume of the lysis solution and/or wherein the cell lysate is mixed with an equal volume of the stabilization solution.

Disclosed are kits for amplifying a whole genome, the kits comprising a lysis

25     solution, a stabilization solution, a set of primers, and a DNA polymerase.

Disclosed are kits wherein the lysis solution comprises potassium hydroxide, such as 400 mM KOH.

Also disclosed are kits wherein the lysis solution comprises 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA.

30     Disclosed are kits, wherein the stabilization solution comprises Tris-HCl at pH 4.1, wherein the stabilization solution comprises 800 mM Tris-HCl, pH 4.1, or wherein the stabilization solution consists of 800 mM Tris-HCl, pH 4.1.

Disclosed are kits comprising deoxynucleotide triphosphates.

Disclosed are kits comprising 1M dithiotheitol, 1X Phosphase-Buffered Saline, pH
7.5, and control DNA template, wherein the lysis solution comprises 400 mM KOH and 10
mM EDTA, wherein the stabilization solution comprises 800 mM Tris-HCl, pH 4, wherein
the set of primers comprises a reaction mix, wherein the reaction mix comprises 150 mM
5      Tris-HCl, 200 mM KCl, 40 mM $MgCl_2$, 20 mM $(NH_4)_2SO_4$, 4 mM deoxynucleotide
triphosphates, and 0.2 mM random hexamer primers, wherein the DNA polymerase is $\phi29$
DNA polymerase.

Disclosed are kits comprising one or more detection probes, wherein the detection
probes each comprise a complementary portion, wherein the complementary portion is
10     complementary to a nucleic acid sequence of interest.

Disclosed are kits, wherein the kit is designed to detect nucleic acid sequences of
interest in the genome and/or kits wherein the kit is designed to assess a disease, condition
or predisposition of an individual based on the nucleic acid sequences of interest.

Disclosed are methods of amplifying damaged DNA, the method comprising
15     exposing a damaged DNA sample to conditions that promote substantial denaturation of
damaged DNA in the damaged DNA sample, thereby forming a denatured damaged DNA
sample, altering the conditions to conditions that do not promote substantial denaturation of
damaged DNA in the damaged DNA sample to form a stabilized damaged DNA sample,
incubating damaged DNA in the stabilized damaged DNA sample under conditions that
20     promote replication of the damaged DNA, wherein replication of the damaged DNA results
in a longer average fragment length for the replicated damaged DNA than the average
fragment length in the damaged DNA sample, wherein during replication at least one of the
replicated strands is displaced by strand displacement replication of another replicated
strand.

25     Disclosed are methods, wherein the damaged DNA sample, the denatured damaged
DNA sample, or both are exposed to ionic conditions, wherein the damaged DNA sample
and denatured damaged DNA sample are exposed to ionic conditions by mixing an ionic
solution with the damaged DNA sample, wherein the ionic solution is mixed with the
damaged DNA sample prior to or during exposure of the damaged DNA sample to
30     conditions that promote substantial denaturation of the damaged DNA, wherein the ionic
solution is a salt solution, wherein the salt solution comprises one or more salts, wherein the
salt is Tris-HCl, Tris-EDTA, soduim chloride, potassium chloride, magnesium chloride,
sodium acetate, potassium acetate, magnesium acetate, or a combination, wherein the Tris-

HCl is from pH 7.0 to 8.0, wherein the salt is Tris-EDTA, wherein the salt solution comprises about 50 mM to about 500 mM Tris and about 1 mM to about 5 mM EDTA, wherein the ionic solution is diluted 2 to 5 fold when mixed with the damaged DNA sample, wherein the denatured damaged DNA sample is exposed to ionic conditions by

5      mixing an ionic solution with the denatured damaged DNA sample, wherein the ionic solution is mixed with the denatured damaged DNA sample prior to or during altering of the conditions, wherein the damaged DNA sample is exposed to conditions that promote substantial denaturation by mixing the damaged DNA sample with a denaturing solution and by heating the damaged DNA sample to a temperature and for a length of time that

10     substantially denatures the damaged DNA in the damaged DNA sample, wherein the damaged DNA sample is mixed with the denaturing solution after the DNA sample is heated, wherein the damaged DNA sample is mixed with the denaturing solution before the DNA sample is heated, wherein the damaged DNA sample is mixed with the denaturing solution at the same time the DNA sample is heated, wherein the damaged DNA sample is

15     mixed with the denaturing solution during heating of the DNA sample, wherein the damaged DNA sample is mixed with the denaturing solution when heating of the DNA sample begins, and/or wherein mixing the damaged DNA sample with a denaturing solution produces alkaline conditions in the damaged DNA sample

       Disclosed are methods wherein the denaturing solution comprises a base, wherein

20     the base is an aqueous base, wherein the base is sodium hydroxide, potassium hydroxide, potassium acetate, sodium acetate, ammonium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, calcium carbonate, ammonia, aniline, benzylamine, n-butylamine, diethylamine, dimethylamine, diphenylamine, ethylamine, ethylenediamine, methylamine, N-methylaniline, morpholine,

25     pyridine, triethylamine, trimethylamine, aluminum hydroxide, rubidium hydroxide, cesium hydroxide, strontium hydroxide, barium hydroxide, or DBU (1,8-diazobicyclo[5,4,0]undec-7-ene), wherein the base is sodium hydroxide, wherein the denaturing solution comprises about 150 mM to about 1 M NaOH.

       Disclosed are methods, wherein the denaturing solution is 10X concentration,

30     wherein the damaged DNA sample is mixed with the denaturing solution to create a 1X concentration, and/or wherein the alkaline conditions comprise 15 to 50 mM NaOH.

       Disclosed are methods, wherein the damaged DNA in the damaged DNA sample is substantially denatured without further damaging the DNA, wherein the damaged DNA

sample is heated to a temperature of about 70°C or less and for a length of time of about 5 minutes or less, wherein the temperature is about 60°C to about 70°C, wherein the temperature is about 50°C to about 60°C, wherein the temperature is about 40°C to about 50°C, wherein the temperature is about 25°C to about 40°C, wherein the temperature is about 60°C to about 70°C and the length of time is about 3 minutes, and/or wherein the temperature is about 25°C to about 50°C and the length of time is about 5 minutes or more.

Disclosed are methods wherein altering the conditions comprises reducing the pH of and cooling the denatured damaged DNA sample, wherein the temperature to which the damaged DNA sample is heated is maintained during reduction of the pH of the denatured damaged DNA sample, wherein the temperature to which the damaged DNA sample is heated is reduced before reduction of the pH of the denatured damaged DNA sample, and/or wherein the temperature to which the damaged DNA sample is heated is reduced during reduction of the pH of the denatured damaged DNA sample.

Disclosed are methods, wherein cooling the denatured damaged DNA sample is commenced during reduction of the pH of the denatured damaged DNA sample, wherein cooling the denatured damaged DNA sample is commenced when the pH of the denatured damaged DNA sample is reduced.

Disclosed are methods, wherein the pH of the denatured damaged DNA sample is reduced by mixing the denatured damaged DNA sample with a stabilization solution.

Disclosed are methods, wherein the pH of the denatured damaged DNA sample is reduced to the range of about pH 7.5 to about pH 8.0.

Disclosed are methods, wherein the pH of the denatured damaged DNA sample is reduced by mixing the denatured damaged DNA sample with a stabilization solution.

Disclosed are methods, wherein the stabilization solution comprises a buffer, and/or wherein the buffer is phosphate buffer, Good buffer, BES, BICINE, CAPS, EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES, TRICINE, sodium cacodylate, sodium citrate, triethylammonium acetate, triethylammonium bicarbonate, Tris, Bis-tris, or Bis-tris propane.

Disclosed are methods, wherein the stabilization solution comprises 800 mM Tris-HCl, pH 4.1.

Disclosed are methods, wherein the stabilization solution comprises an acid.

Disclosed are methods, wherein the acid is hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid, acetylsalicylic acid, ascorbic acid, carbonic acid, citric acid,

formic acid, nitric acid, perchloric acid, HF, HBr, HI, $H_2S$, HCN, HSCN, HClO, monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, or a carboxylic acid.

Disclosed are methods, wherein the carboxylic acid is ethanoic, propanoic, or butanoic.

Disclosed are methods, wherein the stabilization solution comprises a plurality of acidic agents.

Disclosed are methods, wherein the pH of the denatured damaged DNA sample is reduced to the range of about pH 9.0 to about pH 6.8, about pH 9.0 to about pH 7.5, about pH 9.0 to about pH 8.0, about pH 8.5 to about pH 6.8, about pH 8.5 to about pH 7.5, about pH 8.5 to about pH 8.0, about pH 8.0 to about pH 6.8, about pH 8.0 to about pH 7.5.

Disclosed are methods, wherein the pH of the denatured damaged DNA sample is reduced to about pH 9.0 or less, about pH 8.5 or less, about pH 8.0 or less, about pH 7.5 or less.

Disclosed are methods, wherein the stabilization solution comprises one or more salts.

Disclosed are methods, wherein the salt is Tris-HCl, Tris-EDTA, soduim chloride, potassium chloride, magnesium chloride, sodium acetate, potassium acetate, magnesium acetate, or a combination.

Disclosed are methods, wherein the Tris-HCl is from pH 7.0 to 8.0.

Disclosed are methods, wherein the salt is Tris-EDTA.

Disclosed are methods, wherein the stabilization solution comprises about 50 mM to about 500 mM Tris and about 1 mM to about 5 mM EDTA.

Disclosed are methods, wherein the damaged DNA mixture is cooled at a rate of about 1°C per minute or less.

Disclosed are methods, wherein the damaged DNA mixture is cooled at a rate of about 1% per minute or less.

Disclosed are methods, wherein the damaged DNA mixture is cooled to room temperature or lower from 60°C to 70°C, from 50 to 60°C, from 40°C to 50°C, 30°C to 40°C, 50°C to 70°C.

Disclosed are methods, wherein the denaturing solution comprises one or more salts.

Disclosed are methods, wherein the salt is Tris-HCl, Tris-EDTA, soduim chloride, potassium chloride, magnesium chloride, sodium acetate, potassium acetate, magnesium acetate, or a combination.

Disclosed are methods, wherein the Tris-HCl is from pH 7.0 to 8.0.

Disclosed are methods, wherein the salt is Tris-EDTA.

Disclosed are methods, wherein the denaturing solution comprises about 50 mM to about 500 mM Tris and about 1 mM to about 5 mM EDTA.

Disclosed are methods, wherein the damaged DNA sample is comprised of degraded DNA fragments of genomic DNA.

Disclosed are methods, wherein replication and repair of the damaged DNA is accomplished by incubating the damaged DNA in the presence of a DNA polymerase.

Disclosed are methods, wherein the polymerase is a DNA polymerase that can extend the 3'-ends of the damaged DNA.

Disclosed are methods, wherein the DNA polymerase is φ29 DNA polymerase, BST DNA polymerase, Taq DNA polymerase, a modified form of Taq DNA polymerase, a Reverse Transcriptase, T4 DNA polymerase, T7 DNA polymerase, Pol I DNA polymerase, or a modified form of DNA Polymerase I.

Disclosed are methods, wherein the DNA polymerase is φ29 DNA Polymerase.

Disclosed are methods, wherein the damaged DNA is amplified using a kit, wherein the kit comprises a denaturing solution, a stabilization solution, a set of primers, and a DNA polymerase.

Disclosed are methods, wherein the damaged DNA sample is a cell lysate, wherein the cell lysate is produced by exposing cells to alkaline condition, wherein the cell lysate comprises a whole genome,.

Disclosed are methods, comprising following exposure of the cells to alkaline conditions, exposing a first portion of the cell lysate to conditions that promote substantial denaturation of damaged DNA in the first portion of the cell lysate, wherein reducing the pH of the cell lysate comprises reducing the pH of the first portion of the cell lysate to form a first stabilized cell lysate and reducing the pH of a second portion of the cell lysate to form a second stabilized cell lysate, following reducing the pH of the cell lysate, mixing the second stabilized cell lysate with the first stabilized cell lysate under conditions that promote transient denaturation of the ends of damaged DNA in the second stabilized cell lysate and that maintain substantial denaturation of the damaged DNA in the first stabilized cell lysate, thereby forming a stabilized cell lysate mixture, and prior to incubating the stabilized cell lysate, cooling the stabilized cell lysate mixture under conditions that promote annealing of the ends of the transiently denatured damaged DNA to the

substantially denatured damaged DNA, wherein incubating the stabilized cell lysate under conditions that promote replication of the genome also promotes replication of the damaged DNA, wherein the annealed ends of the damaged DNA prime replication, wherein replication of the damaged DNA results in repair of the replicated strands.

5　　　　　　Disclosed are methods of amplifying damaged DNA, the method comprising exposing a first damaged DNA sample to conditions that promote substantial denaturation of damaged DNA in the first damaged DNA sample, thereby forming a denatured damaged DNA sample, reducing the pH of the denatured damaged DNA sample to form a stabilized denatured damaged DNA sample, mixing a second damaged DNA sample with the

10　　stabilized denatured damaged DNA sample under conditions that promote transient denaturation of the ends of damaged DNA in the second sample and that maintain substantial denaturation of the damaged DNA in the stabilized denatured damaged DNA sample, thereby forming a damaged DNA mixture, cooling the damaged DNA mixture under conditions that promote annealing of the ends of the transiently denatured damaged

15　　DNA to the substantially denatured damaged DNA, incubating the annealed damaged DNA under conditions that promote replication of the damaged DNA, wherein the annealed ends of the damaged DNA prime replication, wherein replication of the damaged DNA results in repair of the replicated strands, wherein during replication at least one of the replicated strands is displaced by strand displacement replication of another replicated strand.

20　　　　　Disclosed are methods, wherein the temperature to which the first damaged DNA sample is heated is maintained during mixing of the second damaged DNA sample with the stabilized denatured damaged DNA sample.

　　　　　　Disclosed are methods, wherein the pH of the stabilized denatured damaged DNA sample is not high enough nor low enough to cause further substantial denaturation upon

25　　mixing second damaged DNA sample with the stabilized denatured damaged DNA sample.

　　　　　　Disclosed are methods, wherein the first damaged DNA sample is a portion of a damaged DNA sample, wherein the second damaged DNA sample is a portion of the same damaged DNA sample.

　　　　　　Disclosed are methods, wherein the first damaged DNA sample is from the same

30　　source as the second damaged DNA sample.

　　　　　　Disclosed are methods, wherein the first damaged DNA sample is from the same organism as the second damaged DNA sample.

Disclosed are methods, wherein the first damaged DNA sample is from the same tissue as the second damaged DNA sample.

Disclosed are methods, wherein the second damaged DNA sample is mixed with the stabilized denatured damaged DNA sample at a temperature and for a length of time that transiently denatures the damaged DNA in the second damaged DNA sample.

Disclosed are methods, wherein the temperature is about 70°C or less and the length of time is about 30 seconds or less.

Disclosed are methods, wherein the second damaged DNA sample is mixed with the stabilized denatured damaged DNA sample at a temperature that does not further damaging the DNA.

Disclosed are methods, wherein the temperature is about 60°C to about 70°C, about 50°C to about 60°C, about 40°C to about 50°C, about 25°C to about 40°C, about 25°C to about 70°C. Disclosed are methods wherein the length of time is about 30 seconds.

Also disclosed are methods of amplifying nucleic acids, the method comprising, exposing a sample suspected of containing nucleic acids to alkaline conditions, reducing the pH of all or a portion of the sample to form a stabilized sample, and incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample. Replication of the nucleic acids results in replicated strands, wherein during replication at least one of the replicated strands is displaced from nucleic acids in the sample by strand displacement replication of another replicated strand, wherein the replicated strands have low amplification bias.

Also disclosed are methods of amplifying nucleic acids, the method comprising, exposing a sample that may comprise nucleic acids to alkaline conditions, wherein the alkaline conditions promote lysis of cells that may be present in the sample, reducing the pH of all or a portion of the sample to form a stabilized sample, and incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample. Replication of the nucleic acids results in replicated strands, wherein during replication at least one of the replicated strands is displaced from nucleic acids in the sample by strand displacement replication of another replicated strand, wherein the replicated strands have low amplification bias.

The concentration of nucleic acids in the amplification mixture can favor hybridization of primers over reassociation of the nucleic acids. The concentration of nucleic acids in the amplification mixture can be 10 ng/µl or less. The concentration of nucleic acids in the amplification mixture can be 8 ng/µl or less, 6 ng/µl or less, 5 ng/µl or less, 4 ng/µl or less, 3 ng/µl or less, 2 ng/µl or less, 1 ng/µl or less, or 0.5 ng/µl or less. The concentration of nucleic acids in the amplification mixture can be 100 ng/µl or less.

The amount of nucleic acids in the amplification mixture can be at or above a threshold that can result in low amplification bias in the replicated strands. The amplification mixture can comprise at least 100 ng of nucleic acid. The amplification sample can comprise at least 150 ng, at least 200 ng, at least 300 ng, at least 400 ng, at least 500 ng, at least 1 mg, at least 2 mg, or at least 3 mg of nucleic acid. The amplification mixture can comprise at least 10 ng of nucleic acid.

The amplification bias of the replicated strands can be less than 20-fold for at least ten target sequences in the sample. The amplification bias of the replicated strands can be less than 10-fold for at least ten target sequences in the sample. The locus representation of the replicated strands can be at least 10% for at least 5 different loci. The locus representation of the replicated strands can be at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% for at least 5 different loci. The locus representation of the replicated strands can be at least 10% for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

The amplification bias of the replicated strands can be less than 50-fold. The amplification bias of the replicated strands can be less than 45-fold, less than 40-fold, less than 35-fold, less than 30-fold, less than 25-fold, less than 20-fold, less than 19-fold, less than 18-fold, less than 17-fold, less than 16-fold, less than 15-fold, less than 14-fold, less than 13-fold, less than 12-fold, less than 11-fold, less than 10-fold, less than 9-fold, less than 8-fold, less than 7-fold, less than 6-fold, less than 5-fold, or less than 4-fold. The amplification bias of the replicated strands can be less than 50-fold for at least 5 different

loci, for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20

5  different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

The sample can comprise cells, wherein the alkaline conditions promote lysis of the cells, wherein the alkaline conditions result in a cell lysate. The sample need not be subjected to heating above a temperature or for a time that would cause substantial cell lysis

10  in the absence of the alkaline conditions. The sample can comprise nucleic acids, wherein the nucleic acids comprises a genome, wherein replication of the nucleic acids results in replication of the genome. Replication of the nucleic acids in the sample can result in replication of all or a substantial fraction of the genome. The genome can be a eukaryotic genome, a plant genome, an animal genome, a vertebrate genome, a fish genome, a

15  mammalian genome, a human genome, a bacterial genome, a microbial genome, or a viral genome. The genome can comprise at least 50% of the nucleic acids in the amplification mixture. The genome can comprise at least 90% of the nucleic acids in the amplification mixture. The genome can comprise at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%,

20  at least 98%, or at least 99% of the nucleic acids in the amplification mixture.

The nucleic acids can comprise a plurality of genomes, wherein replication of the nucleic acids results in replication of a plurality of the genomes. At least two of the genomes can be genomes of different organisms. At least one genome can be a human genome and at least one genome can be a bacterial genome, viral genome, microbial

25  genome, or pathogen genome. At least one genome can be a eukaryotic genome, a plant genome, an animal genome, a vertebrate genome, a fish genome, a mammalian genome, a human genome, a bacterial genome, a microbial genome, or a viral genome, and at least one genome is a eukaryotic genome, a plant genome, an animal genome, a vertebrate genome, a fish genome, a mammalian genome, a human genome, a bacterial genome, a microbial

30  genome, or a viral genome. The genomes can comprise at least 50% of the nucleic acids in the amplification mixture. The genomes can comprise at least 90% of the nucleic acids in the amplification mixture. The genomes can comprise at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at

265

least 96%, at least 97%, at least 98%, or at least 99% of the nucleic acids in the amplification mixture.

The sample can be a eukaryotic sample, a plant sample, an animal sample, a vertebrate sample, a fish sample, a mammalian sample, a human sample, a non-human

5    sample, a bacterial sample, a microbial sample, a viral sample, a biological sample, a serum sample, a plasma sample, a blood sample, a urine sample, a semen sample, a lymphatic fluid sample, a cerebrospinal fluid sample, amniotic fluid sample, a biopsy sample, a needle aspiration biopsy sample, a cancer sample, a tumor sample, a tissue sample, a cell sample, a cell lysate sample, a crude cell lysate sample, a tissue lysate sample, a tissue culture cell

10   sample, a buccal swab sample, a mouthwash sample, a stool sample, a mummified tissue sample, a forensic sample, an autopsy sample, an archeological sample, an infection sample, a nosocomial infection sample, a production sample, a drug preparation sample, a biological molecule production sample, a protein preparation sample, a lipid preparation sample, a carbohydrate preparation sample, an inanimate object sample, an air sample, a soil sample, a

15   sap sample, a metal sample, a fossil sample, an excavated material sample, a terrestrial sample, an extra-terrestrial sample, or a combination thereof. The sample can be a serum sample or a plasma sample. The sample can be exposed to alkaline conditions by mixing the sample with a lysis solution. The lysis solution can comprise a base, a buffer, or a combination. The lysis solution can comprise a base, wherein the base is potassium

20   hydroxide. The lysis solution can comprise 400 mM KOH. The lysis solution can comprise 400 mM KOH and 10 mM EDTA. The lysis solution can comprise 100 mM KOH. The lysis solution can comprise 100 mM KOH and 2.5 mM EDTA. The sample can be mixed with an equal volume of the lysis solution.

The pH of the sample can be reduced to the range of about pH 7.0 to about pH 6.8.

25   The pH of the sample can be reduced by mixing the sample with a stabilization solution. The stabilization solution can comprise a buffer, an acid, or a combination. The stabilization solution can comprise a buffer, wherein the buffer is Tris-HCl at pH 4. The stabilization solution can comprises 800 mM Tris-HCl, pH 4. The stabilization solution can comprise 200 mM Tris-HCl, pH 4. The sample can be mixed with an equal volume of the

30   stabilization solution.

Exposing the sample to alkaline conditions, reducing the pH of the sample, and incubating the stabilized sample can be performed in the same reaction chamber. The reaction chamber can comprise a tube, a test tube, an eppendorf tube, a vessel, a micro

266

vessel, a plate, a well, a well of a micro well plate, a well of a microtitre plate, a chamber, a micro fluidics chamber, a micro machined chamber, a sealed chamber, a hole, a depression, a dimple, a dish, a surface, a membrane, a microarray, a fiber, a glass fiber, an optical fiber, a woven fiber, a film, a bead, a bottle, a chip, a compact disk, a shaped polymer, a particle,

5      or a microparticle. The surface can be sealable. The reaction chamber can comprise acrylamide, cellulose, nitrocellulose, glass, gold, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, glass, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, functionalized silane, polypropylfumerate, collagen,

10     glycosaminoglycans, polyamino acids, or a combination. The nucleic acids need not be purified or extracted prior to incubation of the amplification mixture. Nucleic acids in the sample need not be separated from other material in the sample. Nucleic acids in the stabilized sample need not be separated from other material in the stabilized sample.

       The nucleic acids in the stabilized sample can be less than 0.01% pure, less than

15     0.1% pure, less than 0.5% pure, less than 1% pure, less than 5% pure, less than 10% pure, or less than 20% pure by weight excluding water. The sample need not be subjected to substantial purification prior to the incubation. The sample can be subjected to centrifugation, extraction, chromatography, precipitation, filtration, or dialysis prior to the incubation.

20     The method can further comprise detecting the replicated strands. Detecting the replicated strands can comprise detecting the presence, amount, or presence and amount of replicated strands. The presence, amount, or presence and amount of the replicated strands is accomplished by detecting the presence, amount, or presence and amount of one or more target sequences. The amount of a plurality of alleles, loci, or both can be detected.

25     Detection of replicated strands can indicate that the sample contains nucleic acids.

       The replicated strands can be analyzed for allele dropout, wherein there is no allele dropout for 500 or more loci, 400 or more loci, 300 or more loci, 200 or more loci, 100 or more loci, 50 or more loci, 40 or more loci, 30 or more loci, 20 or more loci, 15 or more loci, 10 or more loci, 8 or more loci, 6 or more loci, 5 or more loci, 4 or more loci, 3 or

30     more loci, 2 or more loci, or 1 or more loci. The replicated strands can be analyzed for allele dropout, wherein allele dropout is less than 5% for 500 or more loci, 400 or more loci, 300 or more loci, 200 or more loci, 100 or more loci, 50 or more loci, 40 or more loci, 30 or

more loci, 20 or more loci, 15 or more loci, 10 or more loci, 8 or more loci, 6 or more loci, 5 or more loci, 4 or more loci, 3 or more loci, 2 or more loci, or 1 or more loci.

Also disclosed are methods of detecting the presence of nucleic acids in a sample, the method comprising, exposing the sample to alkaline conditions, reducing the pH of the

5    sample to form a stabilized sample, and incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample, wherein replication of the nucleic acids results in replicated strands, and detecting the replicated strands, wherein detection of replicated strands indicates that the sample comprises nucleic

10   acids.

Also disclosed are methods of detecting the presence of nucleic acids, the method comprising, exposing a sample that may comprise nucleic acids to alkaline conditions, wherein the alkaline conditions promote lysis of cells that may be present in the sample, reducing the pH of the sample to form a stabilized sample, and incubating an amplification

15   mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample, wherein replication of the nucleic acids results in replicated strands, and detecting the replicated strands, wherein detection of replicated strands indicates that the sample comprises nucleic acids.

20   The method can further comprise quantitating the replicated strands. The quantity of replicated strands can be a measure of the amount of nucleic acids present in the sample. The sample can comprise nucleic acids from more than two organisms, wherein detecting the replicated strands detects at least one organism. The sample can comprise nucleic acids from a whole ecosystem, wherein detecting the replicated strands detects at least one

25   organism.

Also disclosed are methods of detecting the presence of an organism in a sample, the method comprising, exposing the sample to alkaline conditions, reducing the pH of the sample to form a stabilized sample, incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification

30   mixture comprises all or a portion of the stabilized sample, wherein replication of the nucleic acids results in replicated strands, and identifying one or more types of organism that contain one or more of the sequences of the replicated strands, thereby detecting the presence of the organism in the sample.

268

The one or more types of organism that contain one or more of the sequences of the replicated strands can be identified by sequencing at least a portion of the replicated strands to obtain one or more sequences of the replicated strands, searching a database of nucleic acid sequences sequentially using one or more of the sequences of the replicated strands as

5      strings, and identifying the results of the search as sequences of organisms likely to be present in the sample and not likely to be present in the sample, thereby detecting the presence of the organism in the sample. The sample can comprise nucleic acids from a whole ecosystem, wherein at least one organism in the sample can be a variant organism, wherein the variant organism can comprise a variant sequence from sequence of the same

10     type of organism present in the database, wherein identifying a type of organism that contains one or more of the sequences of the replicated strands identifies at least one organism. Sequencing at least a portion of the replicated strands can be accomplished by hybridization to at least one of a plurality of nucleic acid probes. The nucleic acid probes are immobilized on a microarray. The sample can comprise nucleic acids from two

15     organisms, wherein identifying a type of organism that contains one or more of the sequences of the replicated strands identifies both organisms. The sample can comprise nucleic acids from two organisms, wherein identifying a type of organism that contains one or more of the sequences of the replicated strands identifies at least one organism.

The sample can be a substantially cell-free sample. The sample can be a serum

20     sample or a plasma sample. The sample can be a water sample. During replication at least one of the replicated strands can be displaced from nucleic acids in the sample by strand displacement replication of another replicated strand. The nucleic acids can be replication using exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification

25     (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), amplification with Qβ replicase, and cycle sequencing.

Also disclosed are methods of amplifying nucleic acids, the method comprising, exposing a sample to alkaline conditions, reducing the pH of all or a portion of the sample

30     to form a stabilized sample, and incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample. Replication of the nucleic acids results in replicated strands, wherein the replicated strands have low amplification bias, wherein

the concentration of nucleic acids in the amplification mixture favors hybridization of primers over reassociation of the nucleic acids, wherein amount of nucleic acids in the amplification mixture is at or above a threshold that can result in low amplification bias in the replicated strands.

5        Also disclosed are methods of amplifying nucleic acids, the method comprising, exposing a sample that may comprise nucleic acids to alkaline conditions, wherein the alkaline conditions promote lysis of cells that may be present in the sample, reducing the pH of all or a portion of the sample to form a stabilized sample, and incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample,

10       wherein the amplification mixture comprises all or a portion of the stabilized sample. Replication of the nucleic acids results in replicated strands, wherein the replicated strands have low amplification bias, wherein the concentration of nucleic acids in the amplification mixture favors hybridization of primers over reassociation of the nucleic acids, wherein amount of nucleic acids in the amplification mixture is at or above a threshold that can

15       result in low amplification bias in the replicated strands.

         The nucleic acids can be replication using exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain

20       reaction (PCR), self-sustained sequence replication (3SR), amplification with Qβ replicase, and cycle sequencing.

         Also disclosed are methods of identifying reaction conditions for nucleic acid amplification, the method comprising amplifying a test sample under test conditions to produce amplified nucleic acids, measuring amplification bias in the amplified nucleic

25       acids, wherein if the amplification bias is less than a threshold of interest, then the test conditions are identified as conditions for a nucleic acid amplification.

         The nucleic acids can be replicated using exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based

30       amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), amplification with Qβ replicase, and cycle sequencing.

270

Also disclosed are methods of identifying reaction conditions for nucleic acid amplification, the method comprising exposing a test sample to alkaline conditions, reducing the pH of all or a portion of the test sample to form a stabilized test sample, and incubating a test amplification mixture under conditions to produce amplified nucleic acids,

5    wherein the test amplification mixture comprises all or a portion of the test stabilized sample, wherein the test conditions promote replication of nucleic acids, wherein the concentration of nucleic acids in the test amplification mixture is a test nucleic acid concentration, wherein the amount of nucleic acids in the test amplification mixture is a test amount of nucleic acids, and measuring amplification bias in the amplified nucleic acids,

10   wherein if the amplification bias is less than a threshold of interest, then the test concentration of nucleic acids is a concentration to be used for nucleic acid amplification and the test amount of nucleic acids is threshold amount to be used for nucleic acid amplification.

Also disclosed are methods of identifying reaction conditions for nucleic acid

15   amplification, the method comprising exposing a test sample to alkaline conditions, wherein the alkaline conditions promote lysis of cells that may be present in the sample, reducing the pH of all or a portion of the test sample to form a stabilized test sample, and incubating a test amplification mixture under conditions to produce amplified nucleic acids, wherein the test amplification mixture comprises all or a portion of the test stabilized sample, wherein

20   the test conditions promote replication of nucleic acids, wherein the concentration of nucleic acids in the test amplification mixture is a test nucleic acid concentration, wherein the amount of nucleic acids in the test amplification mixture is a test amount of nucleic acids, and measuring amplification bias in the amplified nucleic acids, wherein if the amplification bias is less than a threshold of interest, then the test concentration of nucleic acids is a

25   concentration to be used for nucleic acid amplification and the test amount of nucleic acids is threshold amount to be used for nucleic acid amplification.

Also disclosed are methods of amplifying nucleic acids, the method comprising, exposing a sample to alkaline conditions, reducing the pH of all or a portion of the sample to form a stabilized sample, and incubating an amplification mixture under conditions that

30   promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample. Replication of the nucleic acids results in replicated strands, wherein during replication at least one of the replicated strands is displaced from nucleic acids in the sample by strand displacement replication of another

271

replicated strand, wherein the replicated strands have an amplification bias less than a threshold of interest, wherein the concentration of nucleic acids in the amplification mixture is at or above a predetermined concentration, wherein amount of nucleic acids in the amplification mixture is at or above a threshold amount. The predetermined concentration

5      and the threshold amount are determined by exposing a test sample to the alkaline conditions, reducing the pH of all or a portion of the test sample to form a stabilized test sample, and incubating a test amplification mixture under conditions that promote replication of nucleic acids to produce amplified nucleic acids, wherein the amplification mixture comprises all or a portion of the stabilized sample, wherein the concentration of

10     nucleic acids in the test amplification mixture is a test nucleic acid concentration, wherein the amount of nucleic acids in the test amplification mixture is a test amount of nucleic acids, and measuring amplification bias in the amplified nucleic acids, wherein if the amplification bias is less than the threshold of interest, then the test concentration of nucleic acids is the predetermined concentration and the test amount of nucleic acids is the

15     threshold amount.

Also disclosed are methods of amplifying nucleic acids, the method comprising, exposing a sample that may comprise nucleic acids to alkaline conditions, wherein the alkaline conditions promote lysis of cells that may be present in the sample, reducing the pH of all or a portion of the sample to form a stabilized sample, and incubating an amplification

20     mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample. Replication of the nucleic acids results in replicated strands, wherein during replication at least one of the replicated strands is displaced from nucleic acids in the sample by strand displacement replication of another replicated strand, wherein the replicated strands have an

25     amplification bias less than a threshold of interest, wherein the concentration of nucleic acids in the amplification mixture is at or above a predetermined concentration, wherein amount of nucleic acids in the amplification mixture is at or above a threshold amount. The predetermined concentration and the threshold amount are determined by exposing a test sample to the alkaline conditions, reducing the pH of all or a portion of the test sample to

30     form a stabilized test sample, and incubating a test amplification mixture under conditions that promote replication of nucleic acids to produce amplified nucleic acids, wherein the amplification mixture comprises all or a portion of the stabilized sample, wherein the concentration of nucleic acids in the test amplification mixture is a test nucleic acid

concentration, wherein the amount of nucleic acids in the test amplification mixture is a test amount of nucleic acids, and measuring amplification bias in the amplified nucleic acids, wherein if the amplification bias is less than the threshold of interest, then the test concentration of nucleic acids is the predetermined concentration and the test amount of

5       nucleic acids is the threshold amount.

Also disclosed are kits for amplifying a whole genome, the kit comprising a stabilization solution, a reaction mix comprising a set of primers, and a DNA polymerase mix comprising a DNA polymerase. The kit can further comprise 1M dithiotheitol, 1X Phosphase-Buffered Saline, pH 7.5, and control DNA template, where the stabilization

10      solution comprises 800 mM Tris-HCl, pH 4, where the reaction mix comprises 150 mM Tris-HCl, 200 mM KCl, 40 mM MgCl$_2$, 20 mM (NH$_4$)$_2$SO$_4$, 4 mM deoxynucleotide triphosphates, and 0.2 mM random hexamer primers, and where the DNA polymerase is $\phi$29 DNA polymerase.

The sample need not be heated above a temperature or for a time that would cause

15      substantial cell lysis in the absence of the alkaline conditions. The nucleic acids need not be purified or extracted between exposing the sample to alkaline conditions, reducing the pH of the sample, and incubating the stabilized sample. The substantial purification need not include centrifugation, extraction, chromatography, precipitation, filtration, or dialysis. The substantial purification need not include centrifugation, phenol-chloroform extraction, or

20      column chromatography. The sample can be subjected to centrifugation, phenol-chloroform extraction, column chromatography, or a combination of these prior to the incubation. The substantial purification can comprise centrifugation, extraction, chromatography, filtration, dialysis, or a combination of these. The substantial purification can comprise precipitation other than precipitation caused by the alkaline conditions or by the reduction of the pH. The

25      substantial purification can comprise centrifugation, phenol-chloroform extraction, column chromatography, or a combination of these.

The sample can be exposed to alkaline conditions by mixing the sample with a lysis solution. The lysis solution can comprise a base. The base can be an aqueous base. The base can be potassium hydroxide, sodium hydroxide, potassium acetate, sodium acetate,

30      ammonium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, calcium carbonate, ammonia, aniline, benzylamine, n-butylamine, diethylamine, dimethylamine, diphenylamine, ethylamine, ethylenediamine, methylamine, N-methylaniline, morpholine, pyridine, triethylamine, trimethylamine,

aluminum hydroxide, rubidium hydroxide, cesium hydroxide, strontium hydroxide, barium hydroxide, or DBU (1,8-diazobicyclo[5,4,0]undec-7-ene).

The base can be potassium hydroxide. The lysis solution can comprise 400 mM KOH. The lysis solution can comprise 400 mM KOH and 10 mM EDTA. The lysis solution can consist of 400 mM KOH and 10 mM EDTA. The lysis solution can comprise a plurality of basic agents. The sample can be mixed with an equal volume of the lysis solution. The lysis solution can comprise a buffer. The buffer can be a phosphate buffer, Good buffer, BES, BICINE, CAPS, EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES, TRICINE, sodium cacodylate, sodium citrate, triethylammonium acetate, triethylammonium bicarbonate, Tris, Bis-tris, or Bis-tris propane. The lysis solution can comprise a plurality of buffering agents.

The pH of the sample can be reduced to the range of about pH 7.0 to about pH 6.8. The pH of the sample can be reduced by mixing the sample with a stabilization solution. The stabilization solution can comprise a buffer. The buffer can be phosphate buffer, Good buffer, BES, BICINE, CAPS, EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES, TRICINE, sodium cacodylate, sodium citrate, triethylammonium acetate, triethylammonium bicarbonate, Tris, Bis-tris, or Bis-tris propane. The buffer can be Tris-HCl at pH 4. The stabilization solution can comprise 800 mM Tris-HCl, pH 4. The stabilization solution can consist of 800 mM Tris-HCl, pH 4. The stabilization solution can comprise a plurality of buffering agents. The sample can be mixed with an equal volume of the stabilization solution.

The stabilization solution can comprise an acid. The acid can be hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid, acetylsalicylic acid, ascorbic acid, carbonic acid, citric acid, formic acid, nitric acid, perchloric acid, HF, HBr, HI, $H_2S$, HCN, HSCN, HClO, monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, or a carboxylic acid. The carboxylic acid can be ethanoic, propanoic, or butanoic. The stabilization solution can comprise a plurality of acidic agents. The incubation can be substantially isothermic. The sample need not be heated substantially above the temperature of the incubation. The sample need not be subjected to heating above a temperature or for a time that would cause notable denaturation of nucleic acids in the sample.

The method can further comprise exposing a second sample that may comprise nucleic acids to alkaline conditions, wherein the alkaline conditions promote lysis of cells that may be present in the sample, reducing the pH of the sample to form a second stabilized

sample, and incubating the second stabilized sample under conditions that promote

replication of the nucleic acids in the second sample, wherein replication of the nucleic

acids in the second sample results in replicated strands, wherein during replication at least

one of the replicated strands is displaced from nucleic acids in the second sample by strand

5       displacement replication of another replicated strand.

The second sample can be a sample from the same type of organism as the first

sample. The second sample is a sample from the same type of tissue as the first sample.

The second sample is a sample from the same organism as the first sample. The second

sample can be obtained at a different time than the first sample. The second sample can be

10      a sample from a different organism than the first sample. The second sample can be a

sample from a different type of tissue than the first sample. The second sample can be a

sample from a different species of organism than the first sample. The second sample can

be a sample from a different strain of organism than the first sample. The second sample

can be a sample from a different cellular compartment than the first sample.

15                                          **Illustrations**

The following illustrates some specific moses of the disclosed methods.

**High Temperature MDA Protocol Illustration**

Reagent Compositions

Solution A:      400 mM KOH, 10 mM EDTA, pH 8

20      Solution B:      800 mM Tris Hydrochloride, pH 4


1. Denaturation of the genomic DNA template before amplification.

   a. <u>Denaturation Solution</u> Dilute Solution A by 1:4 with $H_2O$ (e.g. 100 μL of

      Solution A into 300 μL of $H_2O$).

25          b. <u>Stabilization Solution</u> Dilute Solution B by 1:5 with $H_2O$ (e.g. 100 μL of

      Solution B into 400 μL of $H_2O$).

Note: Denaturation and Stabilization Solutions should be diluted freshly before use.

The bottle containing Solution A should be stored tightly sealed.

2. Add 2.5 μL of the Denaturation Solution to each 0.2 mL thermocycler tube

30      containing 2.5 μL of genomic DNA on ice. Mix well by pipetting up and down 5 times.

Incubate the tubes or plate on ice for 3 minutes.

3. Stop the denaturation reaction after 3 minutes by adding 5 μL of the Stabilization Solution to each sample and control. Remove the tubes from ice. Proceed immediately to the amplification protocol.

4. Multiple Displacement Amplification

5      To the tube from Step 3, add in a final volume of 50 μl:

Required amount of genomic DNA,

0.3M Trehalose,

10 mM $MgCl_2$,

37.5 mM Tris/HCl pH: 7,

10      50 mM KCl,

20 mM Ammonium Sulfate,

1 mM dNTPs,

50 uM exonuclease-resistant random hexamer oligonucleotide,

40 units of Phi29 DNA polymerase.

15      Incubate at 40 °C for 6-16 hrs.

**Amplification Illustration**

**A. Reagents**

4X Mix

DNA Polymerase Mix

20      Control gDNA Template (10 ng/μL)

Stock Lysis or Denaturation Solution

Stabilization Solution

1X Phosphate-Buffered Saline (pH7.5)

1M Dithiothreitol (DTT)

25      Sterile distilled/deionized water ($H_2O$)

Disodium ethylenediaminetetraacetate-2$H_2O$ (EDTA)

Potassium hydroxide (KOH), pellets

1X TE Buffer (10 mM Tris, pH 8.0; 1 mM EDTA, pH 8.0)

30°C and 65°C Incubators, or thermal cycle controller

30      0.5 μL to 1000 μL micropipettors

1.5 mL polypropylene microcentrifuge tubes

0.2 mL thermal cycler tubes, or 96-well thermal cycler plate

Pipette tips with hydrophobic filter

276

## B. Storage and Stability

Store frozen reagents and components at or below -70°C. Stock Lysis Solution and Denaturing Solution should be prepared fresh and may be stored at room temperature (20-25°C) for about one week to ensure no loss of activity (see below).

## C. Procedures

### 1. Preparation of Stock Lysis Solution

5 mL vial of Stock Lysis Solution – 0.4 M KOH, 10 mM EDTA (pH 8.0) – prepared fresh from stock solutions for use within one week in cell lysis or in purified gDNA denaturation (see below).

Preparation of 5M KOH and 0.5M EDTA Stock Solutions: Tightly resealed stock solutions can be stored at room temperature for up to one year from the date of preparation.

Prepare Stock 5 M KOH by adding 28 g of KOH pellet and sufficient $H_2O$ to make 100 mL final volume. Be sure that the KOH pellets are dry (not clumped) and exposed to air as briefly as possible before dissolution.

Prepare Stock 0.5 M EDTA (pH 8.0) by adding 18.6 g of EDTA to 80 mL of $H_2O$. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH. The disodium salt of EDTA will not go into solution until the pH is adjusted to approximately 8.0. Remove the magnetic stir bar and adjust the final volume to 100 mL. Alternatively, a commercially available molecular biology grade 0.5 M EDTA pH 8.0 can be used.

Preparation of Stock Lysis Solution: Prepare 5 mL of Stock Lysis Solution in a vial by adding 0.4 mL of 5 M KOH and 0.1 mL of 0.5 M EDTA (pH 8.0) into 4.5 mL of sterile distilled/deionized $H_2O$.

Please note: The bottle containing the 5 M KOH stock, and the vial containing Stock Lysis Solution should both be tightly sealed immediately to avoid neutralization from $CO_2$. The vial of Stock Lysis Solution can be stored at room temperature (20-25°C) for about one week to ensure no loss of activity.

### 2. Extraction Protocols

#### i. Fast genomic DNA extraction from tissue culture cells:

Perform the reaction set up on ice.

Dilute tissue culture cells in 1X PBS to a concentration of approximately 100,000 cells/mL.

Dispense 3 µL of each sample of diluted tissue culture cells for amplification into a labeled 0.2 mL thermal cycler tube or 96-well thermal cycler plate, as appropriate.

277

Determine the number of intended reactions, and prepare a sufficient amount of fresh "Lysis Solution" by adding 1/10 volume of 1M Dithiothreitol (DTT) into Stock Lysis Solution (e.g., 3.5 μL of 1M DTT into 31.5 μL of Stock Lysis Solution to prepare 35 μL for 10 reactions). Lysis Solution must be prepared fresh immediately before each new

5    extraction.

Add 3.5 μL of the Lysis Solution to each tube containing 3 μL of tissue culture cells diluted with 1X PBS. Mix well by pipetting up and down 3 times and allow to stand for 10 minutes on ice.

Add 3.5 μL of Stabilization Solution to each tube of lysed tissue culture cells and

10   mix by pipetting up and down 3 times. The 10 μL of lysed tissue culture cells may now be used as template DNA for the amplification reaction in the same 0.2 mL thermal cycler tube (proceed immediately to the Amplification Protocol).

### ii. Fast genomic DNA extraction from blood or buffy coat:

Perform the reaction set up on ice.

15   Dispense 0.5 μL of the human whole blood or buffy coat sample for amplification into a labeled 0.2 mL thermal cycler tube or 96-well thermal cycler plate containing 2.5 μL of 1X Phosphate-Buffered Saline (PBS) for a total of 3 μL.

Determine the number of intended reactions, and prepare a sufficient amount of fresh "Lysis Solution" by adding 1/10 volume of 1M Dithiothreitol (DTT) into Stock Lysis

20   Solution (e.g. 3.5 μL of 1M DTT into 31.5 μL of Stock Lysis Solution to prepare 35 μL for 10 reactions). Lysis Solution must be prepared fresh immediately before each new extraction.

Add 3.5 μL of the Lysis Solution to each tube containing 3 μL of blood or buffy coat diluted with 1X PBS. Mix well by pipetting up and down 3 times and allow to stand

25   for 10 minutes on ice.

Add 3.5 μL of Stabilization Solution to each tube of lysed blood or buffy coat cells and mix by pipetting up and down 3 times. The 10 μL of lysed blood or buffy coat cells may now be used as template DNA for the amplification reaction in the same 0.2 mL thermal cycler tube (proceed immediately to the Amplification Protocol).

30   ### iii. Preparation of Previously Purified gDNA as Amplification Template:

Perform all reaction set up at room temperature.

Set up the reaction tubes containing the appropriate amount of TE and genomic DNA template to give a volume of 2.5 μL before proceeding to the next steps. Determine

278

the volume of genomic DNA to be used, and dispense appropriate amounts of 1X TE buffer into labeled 0.2 mL thermal cycler tubes, or a 96-well thermal cycler plate to bring the total volume of each sample to 2.5 μL. For example, if 1 μL of gDNA template is to be used, add 1.5 μL of 1X TE buffer first and then 1 μL of gDNA template. Include in the sample

5    set a reaction mix containing 10 ng (1 μL) of the Control gDNA Template supplied, as a positive control. The yield should be 45 μg ± 10% for the 50 μL reaction.

Denaturation of the genomic DNA template before amplification. Prepare fresh "Denaturation Solution" by diluting Stock Lysis Solution 1:8 with $H_2O$ (e.g., 5 μL of Stock Lysis Solution into 35 μL of $H_2O$). Prepare fresh "Dilute Stabilization Solution" by diluting

10   Stabilization Solution 1:10 with $H_2O$ (e.g., 5 μL of Stabilization Solution into 45 μL of $H_2O$). Denaturation Solution and Dilute Stabilization Solution should be prepared fresh before each new experiment and must remain at room temperature for proper activity. Denaturation Solution and Dilute Stabilization Solution should not be stored on ice.

Denaturation Reaction. Add 2.5 μL of the Denaturation Solution to each thermal

15   cycler tube or well containing 2.5 μL of sample or control genomic DNA at room temperature. Mix well by pipetting up and down 3 times. Incubate the tubes or plate at room temperature for 3 minutes.

Stop the denaturation reaction after 3 minutes by adding 5 μL of the Dilute Stabilization Solution to each sample and control tube or well. You should now have 10 μL

20   of denatured sample or control solution in each tube or well (proceed immediately to the Amplification Protocol).

### 3. Amplification Protocol

Amplification reaction for isolated gDNA or lysed blood and cells:

Thaw all kit components except the DNA polymerase mix (which should be thawed

25   on ice just prior to use) and then maintain on ice throughout the procedure. The 4X Mix may form a precipitate when it is thawed. The precipitate will fully dissolve upon vortexing for 10 seconds.

Prepare the amplification "Reaction Master Mix". If only a single reaction is to be carried out, the mix may be assembled in a 0.2 mL tube on ice (see Table 1 below for one

30   reaction). For multiple samples, use reagent volumes in the ratios provided in Table 1 below under the heading: Volume (μL) per 10 Reactions. Assemble the Reaction Master Mix in a 1.5 mL microcentrifuge tube on ice (for each 10 samples, prepare sufficient Reaction Master Mix for 11 reactions to provide for residual fluid loss). Add the

components in the order listed in Table 1. After the addition of H$_2$O and 4X Mix, briefly vortex and spin down the mixture before the addition of the DNA Polymerase Mix. Keep the "Reaction Master Mix" on ice.

5    The DNA Polymerase Mix should be thawed on ice just prior to addition to the Reaction Master Mix. The Master Mix should be kept on ice and used for amplification within 30 minutes of the addition of DNA Polymerase Mix.

**Table 1. Reaction Master Mix Preparation:**

| REAGENT | Volume (µL) per Reaction | Volume (µL) per 10 Reactions |
|---|---|---|
| Sterile, Distilled H$_2$O | 27 | 297 |
| 4X Mix | 12.5 | 137.5 |
| DNA Polymerase | 0.5 | 5.5 |
| **TOTAL VOLUME** | **40** | **440** |

10    Set the tubes or plate containing 10 µL of denatured genomic DNA or lysed cells on the bench at room temperature. Add 40 µL of the Reaction Master Mix to each sample tube or plate. Mix well by pipetting up and down 3 times and briefly spin down the mixture.

Immediately transfer the tubes or plates to a 30°C incubator (or a thermal cycle controller set to hold at 30°C) and begin the overnight (approximately 16 hours) incubation.

15    If using a thermal cycler, program to terminate the reactions by incubation at 65°C for 3 minutes (to inactivate the enzyme), and then hold the samples at 4°C until they are retrieved. If using a 65°C incubator, terminate the reactions by incubation for 10 minutes (to inactivate the enzyme).

Store the amplified DNA product tubes or plate at -20°C until needed.

20    **D. Quality Control**

QC Analysis of the Amplification Products

The following protocols may be used to test the quality of the amplified product.

Quantification of DNA yield. PicoGreen® (Molecular Probes) is a fluorescence-based nucleic acid quantification method that is specific for double-stranded DNA and may

25    be used to quantify the double-stranded DNA product. Since the amplification products contain unused reaction primers, it is important to utilize a DNA quantification method that

is specific for double-stranded DNA. The anticipated yield is 45 μg ± 10% per 50 μL reaction.

Qualitative analysis of amplification products. After the reactions are complete, 0.1 to 1 μg of the product can be analyzed by electrophoresis through a 1.0% agarose gel in

5      TBE buffer (90 mM Tris-borate, pH 8.0, 2 mM EDTA), using appropriate stains (e.g. GelStar® (Cambrex), ethidium bromide, or SYBR Green® (Molecular Probes)), and imaged with a UV-light box. The product should appear as an even distribution of sizes ranging from 2 kb to high molecular weight with an average length of greater than 10 kb.

Quantification of locus representation in amplification products. Quantification of a

10     specific locus can be performed using the TaqMan® assay system, developed by Applied Biosystems Inc. A TaqMan assay can determine the yield of a specific genomic DNA sequence. Two representative TaqMan assays measuring c-JUN (Applied Biosystems, Cat. No. 4318299F or T) and human RFLPO (large ribosomal protein) (Applied Biosystems, Cat. No. 4310879E) have been used in evaluation of amplification performance. For

15     RFLPO and c-JUN, the locus representation, defined as the ratio between the copy number of a certain locus in amplified versus template gDNA, should lay between 40 and 200%, depending on the quality of the starting template human gDNA.

## Examples

### A. Example 1:  Whole Genome Amplification using Six Nucleotide Primers

20     This example describes a demonstration of several embodiments of the disclosed method and analysis and comparison of the results. The exemplified method is the disclosed multiple displacement amplification form of whole genome amplification using hexamer (six nucleotide) primers having specific sequences. Different primers, and different number of primers, were used in the various reactions. All of these examples use

25     only one or a few primers of specific nucleotide sequence to efficiently amplify the whole human genome.

#### 1.  Amplification of Human Genomic DNA by Multiple Displacement Amplification (MDA)

In an example of an embodiment of the disclosed method, 100 μl reactions (in

30     triplicates), assembled in 0.2 ml tubes, contained 10 ng human genomic DNA, 37 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM MgCl₂, 5 mM (NH₄)₂SO₄, 1.0 mM dATP, dTTP, dCTP, and dGTP, 50 μM exonuclease resistant hexamer, and 800 units/ml  Phi 29 DNA polymerase. Reactions were incubated for 16 hours at 30°C and terminated by heating to

65°C for 3 min. MDA reaction yield was determined via PicoGreen quantification using a
lambda DNA standard curve (Molecular Probes, Eugene, OR).

### 2. Quantitative PCR Analysis of Amplification Products

The quality of the amplified DNA was assessed using TaqMan analysis. TaqMan
5    analysis was performed using both ABI 7700 and 7000 sequence detection systems
according to the manufacturer's specifications (Applied Biosystems, Foster City, CA). The
TaqMan reaction consists of 50 µl of 1x Platinum Taq Polymerase Buffer, 5 mM $MgCl_2$, 1
mM of each dNTPs, 1 µl of ROX Reference Dye (Invitrogen Life Technologies, Carlsbad,
CA), 1 Unit of Platinum Taq Polymerase (Invitrogen Life Technologies, Carlsbad, CA), 0.3
10   µM each of forward and reverse PCR primers, 0.25 µM of FAM/TAMRA
fluorescent/quencher probe, and 1 µg of MDA amplified DNA. Purified human genomic
DNA (gDNA) (Promega, Madison, WI) was used to generate a standard curve of 0, 0.001,
0.01, 0.1, and 1 µg gDNA to quantify the MDA amplified DNA. Loci representation
(MDA/gDNA) is reported as a percent and is derived as 100 (loci copy number/µg MDA
15   product)/(loci copy number/µg gDNA). A value of 100% indicates that the loci copy
number for the amplified DNA is equal to the loci copy number for the unamplified
genomic DNA.

### 3. Single Primer

Human genomic DNA was amplified by Multiple Displacement Amplification
20   followed by TaqMan loci analysis as described above. A single six nucleotide Alu-specific
primer (that is, its sequence was derived from an Alu repeat sequence), AluR11
(AGCGAG), was used for the MDA reaction. A total of 47 genetic loci were analyzed (2
loci per chromosome, and one locus from the Y chromosome). As illustrated in Figure 1,
AluR11 efficiently amplified the whole human genome as indicated by locus representation.

### 4. Two Primers

Human genomic DNA was amplified by Multiple Displacement Amplification
followed by TaqMan loci analysis as described above using two specific primers. The two
primer sequences were AGTGGG and AGAGAG. A total of 47 genetic loci were analyzed
(2 loci per chromosome, and one locus from the Y chromosome). As illustrated in Figure 2,
30   the two six nucleotide primers efficiently amplified the whole human genome as indicated
by locus representation.

### 5. Three Primers

Human genomic DNA was amplified by Multiple Displacement Amplification followed by TaqMan loci analysis as described above using three specific primers. The primers were six nucleotide non-Alu primers (that is, their sequence was not derived from an Alu repeat sequence) having the sequences AGCCGG, AGTAGG, and AGTTGG. A total of 47 genetic loci were analyzed (2 loci per chromosome, and one locus from the Y chromosome). As illustrated in Figure 3, the three primers efficiently amplified the whole human genome as indicated by locus representation.

### 6. Four Primers

Human genomic DNA was amplified by Multiple Displacement Amplification followed by TaqMan loci analysis as described above using four specific primers. The primers were six nucleotide primers having the sequences AGGCGG, AGTGGG, AGGGAG, and AGTGAG. A total of 47 genetic loci were analyzed (2 loci per chromosome, and one locus from the Y chromosome). As illustrated in Figure 4, the four primer efficiently amplified the whole human genome as indicated by locus representation.

### 7. Five Primers

Human genomic DNA was amplified by Multiple Displacement Amplification followed by TaqMan loci analysis as described above using five specific primers. In a first example, the primers were six nucleotide primers having the sequences AGTGGG, AGCCAG, AGTTAG, AGTCAG, and AGACAG. A total of 47 genetic loci were analyzed (2 loci per chromosome, and one locus from the Y chromosome). As illustrated in Figure 5, these five primers efficiently amplified the whole human genome as indicated by locus representation.

In a second example, the primers were six nucleotide primers having the sequences AGAGGG, AGGCAG, AGCCAG, AGTCAG, and AGACAG. A total of 47 genetic loci were analyzed (2 loci per chromosome, and one locus from the Y chromosome). As illustrated in Figure 6, these five primers efficiently amplified the whole human genome as indicated by locus representation.

In a third example, the primers were six nucleotide primers having the sequences AGTAGG, AGGTGG, AGGCAG, AGACAG, and AGTGAG. A total of 47 genetic loci were analyzed (2 loci per chromosome, and one locus from the Y chromosome). As illustrated in Figure 7, these five primers efficiently amplified the whole human genome as indicated by locus representation.

In a fourth example, the primers were six nucleotide primers having the sequences AGCGAG, AGGAGG, AGAGGG, AGGGAG, and AGTGAG. One of the primers (AGCGAG) was specific for an Alu sequence. A total of 47 genetic loci were analyzed (2 loci per chromosome, and one locus from the Y chromosome). As illustrated in Figure 8, these five primers efficiently amplified the whole human genome as indicated by locus representation.

### 8. Nine Primers

Human genomic DNA was amplified by Multiple Displacement Amplification followed by TaqMan loci analysis as described above using nine specific primers. The primers were six nucleotide Alu-specific primers, referred to as AluR9H. The name and sequence of all nine Alu-specific primers are shown in Table 2. The asterisks represent phosphothiolate linakges. A total of 10 genetic loci were analyzed. As illustrated in Figure 9, the nine primers efficiently amplified the whole human genome as indicated by locus representation.

**Table 2: "AluR9H" mixture of 9 Alu-specific primers**

| AluR1 | 5' CGG T*G*G 3' |
|---|---|
| AluR3 | 5' CGA G*G*C 3 |
| AluR4 | 5' GCG T*G*G 3' |
| AluR6 | 5' AAT C*G*C 3' |
| AluR8 | 5' CCG A*G*A 3' |
| AluR9 | 5' GAT C*G*C 3' |
| AluR10 | 5' AGA G*C*G 3' |
| AluR11 | 5' AGC G*A*G 3' |
| AluR12 | 5' ACT C*C*G 3' |

### 9. Twelve Primers

Human genomic DNA was amplified by Multiple Displacement Amplification followed by TaqMan loci analysis as described above using twelve specific primers. The primers were six nucleotide Alu-specific primers, referred to as AluR12H. The name and sequence of all twelve Alu-specific primers are shown in Table 3. The asterisks represent phosphothiolate linakges. A total of 47 genetic loci were analyzed (2 loci per chromosome, and one locus from the Y chromosome). As illustrated in Figure 10, the twelve primers efficiently amplified the whole human genome as indicated by locus representation.

### Table 3: "AluR12H" mixture of 12 Alu-specific primers

|        |                   |
|--------|-------------------|
| AluR1  | 5' CGG T*G*G 3'   |
| AluR2  | 5' TCA C*G*C 3'   |
| AluR3  | 5' CGA G*G*C 3    |
| AluR4  | 5' GCG T*G*G 3'   |
| AluR5  | 5' ACT C*G*G 3'   |
| AluR6  | 5' AAT C*G*C 3'   |
| AluR7  | 5' CGG A*G*G 3'   |
| AluR8  | 5' CCG A*G*A 3'   |
| AluR9  | 5' GAT C*G*C 3'   |
| AluR10 | 5' AGA G*C*G 3'   |
| AluR11 | 5' AGC G*A*G 3'   |
| AluR12 | 5' ACT C*C*G 3'   |

B. **Example 2: Measurement of DNA synthesis in MDA reaction carried out at in the absence of any additive at 30°C or in the presence of 0.3 M Trehalose at 40°C.**

MDA reactions were carried out at 30°C with no additive or at 40°C with 0.3 M Trehalose, essentially using the protocol described above for high temperature MDA. The quantity of input template was either 0 ng, 0.3 ng, 3 ng, or 30 ng. After 16 hrs of MDA reaction at described temperature, the total DNA synthesis was quantitated by using picogreen assay. The results are shown in Figure 11.

C. **Example 3: Measurement of DNA synthesis in a 16 hrs. MDA reaction carried out at 40°C in the presence of no additive or 0.3 M Trehalose or 0.4 M Sucrose or 0.4 M Glucose.**

MDA reactions were carried out at 40°C either with no additive or in the presence of 0.3 M Trehalose or 0.4 M Sucrose or 0.4 M Glucose, essentially using the protocol described above for high temperature MDA. The quantity of input template was either 0 ng or 0.003 ng or 0.03 ng or 0.3 ng or 3 ng or 30 ng. After 16 hrs of MDA reaction, the total DNA synthesis was quantitated by using picogreen assay. The results are shown in Figure 12.

D. **Example 4: Whole Genome Amplification using Nuclease Resistant Hexamer Primers**

This example describes a demonstration of an embodiment of the disclosed method and analysis and comparison of the results. The exemplified method is the disclosed

multiple displacement amplification form of whole genome amplification using nuclease resistant random hexamer primers. Some reactions in this example were performed without subjecting the sample to denaturing conditions, a preferred form of the disclosed method. In other reactions, the template DNA was subjected to denaturation prior to amplification.

5      MDA was performed using $\phi$29 DNA polymerase.

### 1. Materials and Methods

#### i. DNA and Enzymes.

A panel of human genomic DNA samples, the Human Variation Panel- Caucasian Panel of 100 (reference number HD100CAU) was obtained from Coriell Cell Repositories.

10     Human genomic DNA was also obtained from Promega Corp. Thiophosphate-modified random hexamer (5'-NpNpNpNp$^s$Np$^s$N-3') was synthesized at Molecular Staging, $\phi$29 DNA polymerase was from Amersham Pharmacia Biotech, and yeast pyrophosphatase was from Boehringer-Mannheim. DNA size markers (100 bp DNA ladder, 1 kb DNA ladder) were from Gibco BRL.

15     #### ii. Amplification of Human Genomic DNA.

Human genomic DNA (300 ng to 0.03 ng, as indicated) was placed into 0.2 ml tubes in a total volume of 50 µl, yielding final concentrations of 25 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM MgCl$_2$, and 100 µM exonuclease-resistant hexamer. A heat-treatment step (that is, exposure to denaturing conditions) to increase primer annealing was included or

20     omitted, as indicated, for individual experiments. Annealing reactions were heated to 95°C for 3 minutes and chilled to 4°C in a PCR System Thermocycler (Perkin Elmer). Reactions were then brought to a final volume of 100 µl, containing final concentrations of 37 mM Tris-HCl, pH 8.0, 50 mM KCl, 10 mM MgCl$_2$, 5 mM (NH$_4$)$_2$SO$_4$, 1.0 mM dNTPs, 1 unit/ml of yeast pyrophosphatase, 50 µM exonuclease-resistant hexamer, and 800 units/ml $\phi$29

25     DNA polymerase. Radioactively labeled α-[$^{32}$P] dCTP, approximately 60 cpm/pmol total dNTPs, was added as indicated. Reactions were incubated for 18 hours at 30°C. Incorporation of acid-precipitable radioactive deoxyribonucleotide product was determined with glass fiber filters. After the reactions were terminated, 3 µl aliquots were cleaved with restriction endonuclease AluI and analyzed by electrophoresis through a 1.0% agarose gel in

30     Tris-borate-EDTA buffer, stained with GelStar (Molecular Probes) or SYBR Green (Molecular Probes), and imaged with a Storm 860 PhosphorImager (APB). Denaturing gel analysis was carried out by electrophoresis through a 1.0% agarose gel in 30 mM NaOH, 1

mM EDTA. The radioactive products in the dried gel were visualized with the Storm 860 PhosphorImager.

### iii. Southern Analysis.

10 μg of whole genome amplified DNA or human genomic DNA controls were digested with EcoRI restriction endonuclease and separated through a 1% agarose gel in 1x TBE buffer. Standard Southern analysis procedure (Southern, Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol*. 98:503-517 (1975)) was performed using a Hybond-N+ membrane (Amersham Pharmacia Biotech, Piscataway, NJ). An exon fragment probe of parathyroid hormone (p20.36) and RFLP marker probes for the D13S12 (p9D11) and Thyroglobulin (pCHT.16/8) loci were obtained from American Type Culture Collection. Probes were radiolabeled using the NEBlot random primer labeling method (New England Biolabs, Beverly, MA). The membrane was prehybridized for 1 hr and hybridized to the radiolabeled probe overnight in a Membrane Hybridization Buffer (Amersham Pharmacia Biotech, Piscataway, NJ). The hybridized membrane was washed in 2x SSC and 0.1% SDS twice for 5 min at room temperature, 1x SSC and 0.1%SDS for 15 min at 42°C, and 0.1x SSC twice for 15 min at 65°C. The membrane was then exposed overnight and analyzed using the Storm 860 PhosphorImager.

### iv. Quantitative PCR Analysis.

TaqMan analysis was performed using the ABI 7700 according to the manufacturer's specifications (Applied Biosystems, Foster City, CA) using 1 μg of amplified DNA as template. TaqMan assay reagents for the 8 loci tested were obtained from ABI. The 8 loci and their chromosome assignments were, acidic ribosomal protein (1p36.13); connexin 40 (1q21.1); chemokine (C-C motif) receptor 1 (3p21); chemokine (C-C motif) receptor 6 (6q27); chemokine (C-C) receptor 7 (17q21); CXCR5 Burkitt lymphoma receptor 1 (chr. 11); c-Jun (1p32-p31); and MKP1 dual specificity phosphatase 1 (5q34). Connexin 40 is located near the centromere and chemokine (C-C motif) receptor 6 is located near the telomere. A standard curve for input template was generated to determine the loci copy number in amplified DNA relative to that of genomic DNA. The standard curve was generated from 0, 0.001, 0.01, 0.1, 0.5, and 1 μg of genomic DNA.

### v. Amplification of Human Genomic DNA by Degenerate Oligonucleotide PCR (DOP-PCR).

Human genomic DNA (ranging from 300 ng to 0.03 ng) was amplified as described (Telenius *et al.*, *Genomics*. 13:718-725 (1992)). Radioactively labeled α-[$^{32}$P] dCTP,

approximately 60 cpm/pmol total dNTPs, was added to the reaction for the quantitation of

PCR product yield. Taq DNA Polymerase was from Invitrogen Life Technologies,

Carlsbad, CA. DOP-PCR amplifications were carried out using a GeneAmp 9700 PCR

System thermocycler (Applied Biosystems, Foster City, CA). Locus representation in the

5    DOP-PCR product was quantitatively analysed using the TaqMan assay (Invitrogen Life

Technologies, Carlsbad, CA).

### vi. Amplification of Human Genomic DNA by Primer Extension Preamplification (PEP).

Human genomic DNA (ranging from 300 ng to 0.03 ng) was placed into 0.2 ml

10   tubes in a total volume of 60 μl, yielding final concentrations of 33 μM PEP random primer

(5'-NNN NNN NNN NNN NNN-3') as described (Zhang *et al.*, Whole genome

amplification from a single cell: implications for genetic analysis. *Proc Natl Acad Sci U S*

*A.* 89:5847-5851 (1992)). Radioactively labeled α-[$^{32}$P] dCTP, approximately 60 cpm/pmol

total dNTPs, was added to the reaction for the quantitation of PCR product. PEP reactions

15   were carried out using a GeneAmp 9700 PCR System thermocycler (Applied Biosystems,

Foster City, CA). Locus representation in the PEP product was quantitatively analysed

using the TaqMan assay (Invitrogen Life Technologies, Carlsbad, CA).

### vii. Genotyping of Single Nucleotide Polymorphisms.

The SNPs analyzed here had the following chromosomal locations; 1822, 251, and

20   221, 13q32; 465, 458, and 474, 19q13; VCAM, 1p31; IL-8, 4q13; PDK2-2, 17p; SNP21,

not known. Assays were carried out as described by Faruqi *et al.*, High-Throughput

Genotyping of Single Nucleotide Polymorphisms with Rolling Circle Amplification. *BMC*

*Genomics*, 2:4 (2001). Briefly, DNA denaturation, annealing and ligation reactions were

carried out in an Eppendorf Master Cycler (Eppendorf Scientific, Germany). Exponential

25   RCA reactions were performed in the Real-Time ABI 7700 Sequence Detector (Perkin

Elmer). Two controls lacking ligase were also carried out for each SNP, confirming the

specificity of the assays. The DNA samples were digested with the restriction endonuclease

AluI before being used as template in the ligation reaction. Ligation reactions were set up

in 96-well MicroAmp Optical plates (Perkin Elmer) in a 10 μl reaction volume containing 1

30   unit Ampligase (Epicentre Technologies), 20 mM Tris-HCl (pH 8.3), 25 mM KCl, 10 mM

MgCl₂, 0.5 mM NAD, and 0.01% Triton® X-100. Standard reactions contained 0.5 pM

open circle padlock and 100 ng of Alu I digested genomic DNA. DNA was denatured by

heating the reactions at 95°C for 3 min followed by annealing and ligation at 60°C for 20

min. 20μl of ERCA mix was added to the 10μl ligation reaction. The ERCA mix contained

5% TMA oxalate, 400 μM dNTP mix, 1 μM each of the two primers, 8 units of Bst

polymerase (New England Biolabs, MA), and 1X modified ThermoPol II reaction buffer

containing 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$ and 0.1% Triton®

5       X-100.

### viii.   Comparative Genome Hybridization.

Genomic DNA preparations were nick-translated to incorporate nucleotides

modified with biotin for amplified samples or digoxigenin for unamplified control samples.

Equimolar amounts of amplified and unamplified DNA were co-hybridized in the presence

10      or absence of CotI DNA to suppress repetitive DNA cross-hybridization. Specific

hybridization signals were detected by avidin-FITC and anti-digoxigenin rhodamine.

Captured images of metaphase chromosomes were analyzed using the Applied Imaging

CGH software program and fluorescence profiles were generated. As controls,

differentially labeled amplified or unamplified DNAs were mixed, hybridized, detected and

15      subjected to ratio analysis as outlined above.

### 2. Results

Using the embodiment of the disclosed method, 30 pg (approximately 9 genomic

copies) of human genomic DNA was amplified to approximately 30 μg within 4 hours. The

average fragment length was greater than 10 kb. The amplified human DNA exhibited

20      normal representation for 10 single nucleotide polymorphisms (SNPs). Maximum bias

among 8 chromosomal loci was less than 3-fold in contrast to four to six orders of

magnitude for PCR-based WGA methods. Human DNA amplified with the disclosed

method is useful for several common methods of genetic analysis, including genotyping of

single nucleotide polymorphisms (SNP), chromosome painting, Southern blotting and

25      RFLP analysis, subcloning, and DNA sequencing.

It has been discovered that the use of random hexamer primers and φ29 DNA

polymerase in multiple displacement amplification, a cascading, strand displacement

reaction (U.S. Patent No. 6,124,120 to Lizardi), will readily amplify linear, human genomic

DNA. Amplification of genomic DNA by the disclosed form of MDA at 30°C is

30      exponential for 4-6 hours. The effect of template concentration on amplification yield in the

disclosed method is shown in Figure 13. 100 fg to 10 ng human genomic DNA was

denatured at 95°C for 5 min, and then MDA was carried out at 30°C as described above.

Aliquots were taken at the times indicated in Figure 13 to quantitate DNA synthesis.

Amplification reactions (100 μl) yielded approximately 25-30 μg DNA product regardless of the starting amount of genomic DNA over a 5-log range (100 fg to 10 ng; Figure 13). For some applications, this allows subsequent genetic analysis without the need to measure or adjust DNA concentration.

5      The products of the MDA reaction were characterized as follows. Radioactively labeled human genome amplification samples (0.6 μg) were electrophoresed through an alkaline agarose gel (1%, Tris-Borate EDTA buffer), stained, and imaged as described above. Average product length exceeded 10 kb.

To examine the integrity of amplified DNA, a restriction fragment within the human
10    parathyroid hormone gene (chromosome 11p15.2-15.1) was analyzed on Southern blots. A 1.9 kb restriction enzyme fragment was observed for MDA-based WGA products amplified from as few as 10 genomic copies (or $10^6$-fold amplification). These MDA reactions included a heat denaturation step and amplification was carried out as described above. EcoRI DNA digests were probed using a radioactively-labeled genomic fragment of the
15    parathyroid hormone gene (p20.36) that hybridized to an approximately 1.9 kb DNA fragment. The EcoRI-cleaved DNA preparations were genomic DNA, DNA amplified by MDA from varying amounts of input genomic DNA, or an MDA reaction that lacked input genomic DNA template. This demonstrates that the products of MDA are long enough to yield specific DNA fragments several kb in length after cleavage by restriction
20    endonucleases.

While PCR-based WGA methods typically generate products of only several hundred nucleotides in length (Telenius et al., Genomics. 13:718-725 (1992); Cheung and Nelson, Proc Natl Acad Sci U S A. 93:14676-14679 (1996); Zhang et al., Proc Natl Acad Sci U S A. 89:5847-5851 (1992)), products of the disclosed method were of sufficient length
25    and integrity for RFLP-based genotyping: 16 random individuals were correctly genotyped by the presence of a 2.1 kb, and 1.1 kb Pst I fragment. Specifically, PstI DNA digests were probed using a radioactively-labeled genomic fragment of the RFLP marker D13S12 locus (p9D11) that hybridized to an invariant 3.8 kb DNA fragment and a polymorphic 2.1 kb (allele A) or 1.1 kb (allele B) DNA fragment. The PstI-cleaved DNA preparations were
30    genomic DNA and 5 different patient DNAs amplified by MDA without any heat denaturation of the DNA template (10,000x amplification).

Omission of denaturation conditions prior to MDA was useful for detection of restriction fragments greater than 5 kb in length. MDA reactions were performed with or

without heat denaturation of the genomic target DNA heterozygous for two thyroglobulin alleles. Amplification was carried out as described above. TaqI DNA digests were probed using a radioactively-labeled genomic fragment of the thyroglobulin gene (pCHT.16/8) that hybridized to invariant 1 kb and 3 kb DNA fragments and a polymorphic 5.8 kb (allele A)

5  or 5.2 kb (allele B) DNA fragment. The TaqI-cleaved DNA preparations were genomic DNA, DNA amplified by MDA reaction (10,000x) with a 95°C preheating step, and an MDA reaction (10,000x) without the preheating step. MDA without heat denaturation gave a good yield of DNA fragments 5.2 and 5.8 kb in size, while neither of the 5.2 or 5.8 kb DNA fragments were visible using MDA with heat denaturation of the template.

10  The most useful results from whole genome amplification are obtained when the amplification provides complete coverage of genomic sequences and minimal amplification bias. It is also preferred that the amplification product perform similarly to unamplified genomic DNA during subsequent genetic analysis. Genome coverage after MDA with heat denaturation of the template was examined for 10 randomly distributed SNPs after

15  amplifications of 100-, 10,000-, or 100,000- fold. The presence of all loci was confirmed in the amplified DNA with the exception of one locus (PDK2-2) in 100,000-fold amplified DNA (Table 4). MDA DNA from 72 individuals was genotyped for one of these SNPs. Following 100-fold amplification by MDA, genotyping accuracy was 97% (70 of 72 genotypes scored correctly, Table 4, SNP 1822), a result that was indistinguishable from

20  unamplified genomic DNA genotyped by the same method. MDA-based WGA thus offers an attractive alternative to multiple locus-specific PCR preamplifications for large SNP scoring studies, especially where sample availability is limited.

Table 4

| SNP designation | Fold whole genome amplification | | |
|---|---|---|---|
| | 100X | 10,000X | 100,000X |
| | Correct SNP calls/total assays | | |
| 1822 | 70/72 | 4/4 | 3/4 |
| 251 | 8/8 | 4/4 | 4/4 |
| 221 | 3/4 | 3/4 | 4/4 |
| 465 | 4/4 | 3/4 | 3/4 |
| 458 | 4/4 | 3/4 | 3/4 |
| 474 | 4/4 | 3/4 | 4/4 |

| VCAM | 4/4 | 4/4 | 4/4 |
|---|---|---|---|
| IL-8 | 4/4 | 4/4 | 3/4 |
| SNP21 | 4/4 | 4/4 | 3/4 |
| PDK2-2 | 4/4 | 4/4 | 0/12 |
| | | | |

Sequence bias can occur in amplification methods, and may result from factors such as priming efficiency, template accessibility, GC content, and proximity to telomeres and centromeres. Amplification bias of the disclosed MDA method was examined by TaqMan quantitative PCR for 8 genes, including one near the centromere of chromosome 1 (connexin 40) and one adjacent to the telomere of chromosome 6 (chemokine (C-C motif) receptor 6). For 100, 1,000, and 10,000-fold MDA without heat denaturation, the maximum amplification biases were only 2.7, 2.3, and 2.8 respectively, expressed as the ratio of the most highly represented gene to the least represented gene. In contrast, two WGA methods based on PCR, DOP-PCR (Telenius et al., Genomics. 13:718-725 (1992); Cheung and Nelson, Proc Natl Acad Sci U S A. 93:14676-14679 (1996)) and PEP (Zhang et al., Proc Natl Acad Sci U S A. 89:5847-5851 (1992)), exhibited amplification bias of 4-6 orders of magnitude. These values were consistent with literature values for the bias of PCR-mediated WGA methods; PEP has been reported to generate an amplification bias of up to 50-fold even between two alleles of the same gene (Paunio et al., Clin. Chem. 42:1382-1390 (1996)). Significantly, the 3-fold amplification bias of MDA remained almost constant between 100 and 100,000-fold amplification. An absence of significant sequence bias observed in MDA-based WGA may be explained by the extraordinary processivity of $\Phi 29$ DNA polymerase (Blanco et al., J. Biol. Chem. 264:8935-8940 (1989)). Tight binding of the polymerase to the template assures replication through obstacles caused by DNA primary or secondary structure.

Surprisingly, TaqMan quantification indicated that certain gene loci were enriched by MDA in amplified DNA; locus representation was >100% of the representation in genomic DNA. Representation of mitochondrial DNA was the same between starting gDNA and amplified DNA. Southern blots and chromosome painting experiments indicated that repetitive sequences were underrepresented in MDA product, conferring an effective enrichment of genes. Thus, amplified DNA contained between 100-300% copy numbers of 8 genes relative to genomic DNA. Additional studies will be necessary to identify the

extent and type of repetitive element under-representation; one hypothesis for this observation is that primers corresponding to highly repetitive elements become depleted during MDA. However, in contrast to PCR-based WGA products, which contain up to 70% amplification artifacts (Cheung and Nelson, Whole genome amplification using a

5    degenerate oligonucleotide primer allows hundreds of genotypes to be performed on less than one nanogram of genomic DNA. *Proc Natl Acad Sci U S A*. 93:14676-14679 (1996)), MDA-based WGA products appear to be entirely derived from genomic sequences.

Whole genome MDA was also tested for uniformity of chromosome coverage by comparative genomic hybridization. MDA-generated DNA was cohybridized to metaphase

10    chromosomes with an equivalent amount of unamplified genomic DNA. Amplification reactions included a heat denaturation step and amplification was carried out as described above. Amplified and unamplified DNA samples were nick-translated to incorporate biotin nucleotide and digoxigenin nucleotide, respectively. Specific signals were detected by avidin FITC and anti-digoxigenin rhodamine. With CotI suppression, the hybridization

15    patterns of MDA probes and unamplified probes were indistinguishable even after 100,000-fold amplification. Without CotI suppression, however, the 100,000-fold amplified probe gave reduced centromeric signals, indicating some loss of repetitive centromeric sequences. The uniformity of signal along the length of the chromosome arms was further evidence that MDA-based WGA does not induce significant amplification bias. These results indicated

20    that MDA compared favorably with DOP PCR for preparation of chromosome painting probes (Kim et al., Whole genome amplification and molecular genetic analysis of DNA from paraffin-embedded prostate adenocarcinoma tumor tissue. *J Urol*. 162:1512-1518 (1999); Klein et al., Comparative genomic hybridization, loss of heterozygosity, and DNA sequence analysis of single cells. *Proc Natl Acad Sci U S A* 96:4494-4499 (1999); Wells et

25    al., Detailed chromosomal and molecular genetic analysis of single cells by whole genome amplification and comparative genomic hybridization. *Nucleic Acids Res* 27:1214-1218 (1999)), but that unlike the latter, suppression hybridization may be unnecessary for detection of single copy sequences. This method should be invaluable for DNA probe preparation for comparative genome hybridization, karyotyping and chip-based genetic

30    analysis from limited patient DNA sources such as needle biopsy material or amniocentesis samples.

For genome subcloning and sequencing MDA appears to have several intrinsic advantages over PCR-based methods. Product size of >10kb is compatible with genome

subcloning. Since no in vivo propagation of amplified material is necessary, MDA may represent an efficient method for amplifying "poisonous" genomic sequences. In addition, φ29 DNA polymerase used for MDA has an error rate of 1 in $10^6 - 10^7$ (Esteban et al., Fidelity of Phi29 DNA polymerase. Comparison between protein-primed initiation and

5       DNA polymerization. *J. Biol. Chem.* 268:2719-2726 (1993)) in contrast to approximately 3 in $10^4$ for PCR with Taq DNA polymerase (Eckert and Kunkel, DNA polymerase fidelity and the polymerase chain reaction. *PCR Methods and Applications.* 1:17-24 (1991)). Therefore, PCR accumulates about one mutation per 900 bases (Saiki *et al.*, Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487-

10      491 (1988)) after 20 cycles. Sequence error rates of cloned MDA-based WGA products appear to be similar to those for cloned genomic DNA. Furthermore, minimal amplification bias, uniform yields, and assay simplicity make MDA amenable to the automated, high-density microwell formats used for genome subcloning and sequencing.

        In summary, the utility of MDA-based WGA was demonstrated for a variety of uses

15      including quantitative PCR, SNP genotyping, Southern blot analysis of restriction fragments, and chromosome painting. Several situations may be contemplated where MDA may represent the method of choice for WGA: Firstly, applications where faithful replication during WGA is necessary, such as molecular cloning, or single cell analysis; Secondly, applications where adequate genome representation during WGA is critical, such

20      as genome-wide SNP genotyping studies; And thirdly, where minimization of bias during WGA is important, particularly cytogenetic testing such as pre-natal diagnosis (Harper and Wells, Recent advances and future developments in PGD. *Prenat Diagn.* 19:1193-1199 (1999)), comparative genome hybridization (Wells and Delhanty, Comprehensive chromosomal analysis of human preimplantation embryos using whole genome

25      amplification and single cell comparative genomic hybridization. *Mol Hum Reprod.* 6:1055-1062 (2000)), and assessment of loss of heterozygosity (Paulson et al., Loss of heterozygosity analysis using whole genome amplification, cell sorting, and fluorescence-based PCR. *Genome Res.* 9:482-491 (1999)). Additional situations where there is a significant need for WGA include amplification of DNA from micro-dissected tissues (Kim

30      et al., Whole genome amplification and molecular genetic analysis of DNA from paraffin-embedded prostate adenocarcinoma tumor tissue. *J Urol.* 162:1512-1518 (1999)), buccal smears (Gillespie *et al.*, HLA class II typing of whole genome amplified mouth swab DNA. *Tissue Antigens.* 56:530-538 (2000)), and archival, anthropological samples (Buchanan *et*

*al.*, Long DOP-PCR of rare archival anthropological samples. *Hum Biol.* 72:911-925
(2000)). Finally, DNA amplified from sorted, individual chromosomes may be used for the
generation of whole chromosome-specific painting probes (Guillier-Gencik et al.,
Generation of whole-chromosome painting probes specific to each chicken

5      macrochromosome. *Cytogenet Cell Genet.* 87:282-285 (1999)).

E.   **Example 5: Increasing Time of Incubation at 95°C Causes Increasing Template
     DNA Strand Breakage.**

         This example demonstrates that significant template DNA strand breakage is
generated by incubation at 95°C (which is used in typical amplification reactions to denature

10     the DNA), and that strand breakage is reduced by decreasing the duration of heat treatment.
As with most nucleic acid amplification techniques, the integrity of the starting DNA
template can have an important effect on the rate and yield of the amplified product. In
reactions where the nucleic acid to be amplified is degraded, the yield of amplified product
may be reduced both in quality and quantity. This example demonstrates the reduction of

15     template DNA strand breakage by decreasing time of incubation at 95°C.

         Six reactions were carried out under the conditions used for DNA template strand
separation with heat-denaturation in order to illustrate the degradation of template DNA.
Human genomic DNA (10 µg) was placed into a 0.2 ml tube in a total volume of 50 µl,
yielding final concentrations of 25 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM MgCl$_2$.

20     Reactions were heated to 95°C in a PCR System Thermocycler (Perkin Elmer), and an
aliquot of 8 µl was taken and put on ice at indicated times. For each time point, a 2 µl
aliquot was analyzed by electrophoresis through a 0.8% alkaline agarose gel (30 mM
NaOH, 1 mM EDTA). After electrophoresis, the gel was neutralized with 1X TBE, stained
with SYBR Green II (Molecular Probes), and imaged with a Storm 860 PhosphorImager

25     (APB). The total amount of DNA imaged was determined for each lane of the gel and the
fragment size at which 50% of the DNA was larger, and 50% was smaller, was determined
for the samples drawn at each time point. The results are shown in Figure 14.

         As can be seen, significant breakage of template DNA occurs after incubation at
95°C for longer than three minutes. Such DNA breakage is substantially reduced when

30     incubation is limited to one minute.

F.   **Example 6:   Increasing Time of DNA Template Incubation at 95°C Causes Decreased Rate and Yield of DNA Amplification.**

This example demonstrates that increased time of template DNA incubation at 95°C (which is used in typical amplification reactions to denature the DNA) causes a reduction in both the rate and the yield with which DNA is amplified by MDA.  Omission of DNA template incubation at 95°C results in the greatest rate and yield of DNA amplification.

Six MDA reactions were carried out using template DNA treated at 95°C under the conditions described in Example 5.  Purified human genomic DNA (3 ng) was placed into 0.2 ml tubes in a total volume of 50 µl, containing 25 mM Tris-HCl, pH 7.5, 50 mM KCl, and 10 mM MgCl$_2$.  Reactions were heated to 95°C for the time indicated and chilled to 4°C in a PCR System Thermocycler (Perkin Elmer).  These reactions were then brought to a final volume of 100 µl, containing final concentrations of 37 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM MgCl$_2$, 50 µM exonuclease-resistant hexamer, 5 mM (NH$_4$)$_2$SO$_4$, 1.0 mM dNTPs, 1 unit/ml of yeast pyrophosphatase, and 800 units/ml φ29 DNA polymerase.  Radioactively labeled α-[$^{32}$P] dCTP, approximately 60 cpm/pmol total dNTPs, was added and reactions were incubated for 22 hours at 30°C.  Aliquots were taken at the times indicated and incorporation of acid-precipitable radioactive deoxyribonucleotide product was determined with glass fiber filters.  The results are shown in Figure 15.

As can be seen, omission of heat treatment of the DNA template results in the optimal rate and yield of DNA synthesis (see 0 min curve).  Increasing duration of DNA template heat treatment resulted in progressively reduced rate and yield of DNA synthesis (see 1 min, 3 min, 5 min, 10 min, and 20 min curves).

G.   **Example 7:   Increasing Time of DNA Template Incubation at 95°C Results in Decreasing DNA Product Strand Size.**

This example demonstrates that increased time of template DNA incubation at 95°C (which is used in typical amplification reactions to denature the DNA) causes a reduction in the length of the DNA products amplified by MDA.  Omission of DNA template incubation at 95°C results in the greatest size of DNA amplification products.

Six MDA reactions were carried out using template DNA treated at 95°C under the conditions described in Example 6.  Radioactively labeled α-[$^{32}$P] dCTP was added, approximately 60 cpm/pmol total dNTPs.  Reactions were incubated for 22 hours at 30°C and aliquots were taken at the times indicated.  For each time point, a 2 µl aliquot was analyzed by electrophoresis through a 0.8% alkaline agarose gel (30 mM NaOH, 1 mM

296

EDTA). After electrophoresis, the gel was neutralized and imaged as described in Example 5. The total amount of DNA imaged was determined for each lane of the gel and the fragment size at which 50% of the DNA was larger, and 50% was smaller, was determined for the samples from each time point. The results are shown in Figure 16.

5      As can be seen, omission of heat treatment of the DNA template results in the synthesis of the largest DNA products. Increasing duration of DNA template heat treatment resulted in progressively reduced DNA product size.

H.  **Example 8:  Omission of DNA Template Incubation at 95°C Results in Increased Locus Representation in DNA Products Amplified by MDA.**

10     This example demonstrates that omission of template DNA incubation at 95°C (which is used in typical amplification reactions to denature the DNA) results in no loss in the representation of eight randomly selected loci in DNA products amplified by MDA. Omission of DNA template incubation at 95°C actually results in an increase in locus representation of DNA amplification products relative to template genomic DNA.

15     Two MDA reactions were carried out using template DNA either treated or not treated at 95°C. Purified human genomic DNA (3 ng) was placed into a 0.2 ml tube in a total volume of 50 µl, containing 25 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM MgCl$_2$, and 100 µM exonuclease-resistant hexamer. The annealing reaction was heated to 95°C for 3 minutes and chilled to 4°C in a PCR System Thermocycler (Perkin Elmer). The reaction

20     was then brought to a final volume of 100 µl, containing final concentrations of 37 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM MgCl$_2$, 50 µM exonuclease-resistant hexamer, 5 mM (NH$_4$)$_2$SO$_4$, 1.0 mM dNTPs, 1 unit/ml of yeast pyrophosphatase, and 800 units/ml φ29 DNA polymerase. For amplification lacking the heat denaturation step, DNA template (3 ng) was placed directly into a 0.2 ml tube in a total volume of 100 µl containing 37 mM

25     Tris-HCl, pH 7.5, 50 mM KCl, 10 mM MgCl$_2$, and 50 µM exonuclease-resistant hexamer, 5 mM (NH$_4$)$_2$SO$_4$, 1.0 mM dNTPs, 1 unit/ml of yeast pyrophosphatase, and 800 units/ml φ29 DNA polymerase. Reactions were incubated for 18 hours at 30°C.

TaqMan® quantitative PCR analysis was performed using the ABI 7700 according to the manufacturer's specifications (Applied Biosystems, Foster City, CA) using 1 µg of

30     MDA-amplified DNA as template. TaqMan® assay reagents for the 8 loci tested were obtained from ABI. The 8 loci and their chromosome assignments were, acidic ribosomal protein (1p36.13); connexin 40 (1q21.1); c-Jun (1p32-p31); MKP1 dual specificity phosphatase 1 (5q34); chemokine (C-C motif) receptor 7 (17q21); chemokine (C-C motif)

receptor 1 (3p21); CXCR5 Burkitt lymphoma receptor 1 (chr. 11); and chemokine (C-C motif) receptor 6 (6q27). Connexin 40 is located near the centromere and chemokine (C-C motif) receptor 6 is located near the telomere. A standard curve for input template was generated to determine the loci copy number in amplified DNA relative to that of genomic

5  DNA. The standard curve was generated from 0, 0.001, 0.01, 0.1, 0.5, and 1 µg of genomic DNA. The locus representation was expressed as a percent, relative to the locus representation in the input genomic DNA (such a locus representation can be referred to as a locus representation bias), and was calculated as the yield of quantitative PCR product from 1 µg amplified DNA divided by the yield from 1 µg genomic DNA control. The results are

10  shown in Figure 17.

As can be seen, there is no reduction of locus representation in DNA amplified from template DNA without heat treatment at 95°C. However, significant loss of locus representation was observed from template DNA heat-denatured for 3 min at 95°C.

I.  **Example 9:  Amplification Bias of Loci Amplified by MDA is Significantly Lower**

15  **than Amplification Bias of DNA Amplified by PEP or DOP-PCR.**

This example demonstrates that, for 100-, 1,000-, and 10,000-fold MDA-amplified DNA, omission of template DNA incubation at 95°C (which is used in typical amplification reactions to denature the DNA) results in low bias in the representation of eight randomly selected loci in DNA products. In contrast, two whole genome amplification (WGA)

20  methods based on PCR, DOP-PCR and PEP, exhibit amplification biases of 2-6 orders of magnitude.

MDA reactions were carried out using template DNA not treated at 95°C as described in Example 8. Reactions (100 µl) contained 300, 30, 3, or 0.3 ng DNA, resulting in approximately 100-, 1000-, 10,000-, and 100,000-fold DNA amplification.

25  Amplification of human genomic DNA by degenerate oligonucleotide PCR (DOP-PCR; Telenius et al., *Genomics*. 13:718-725 (1992); Cheung and Nelson, *Proc Natl Acad Sci U S A*. 93:14676-14679 (1996)) was carried out as follows. Human genomic DNA (ranging from 300ng to 0.03ng) was placed into 0.2 ml tubes in a total volume of 50 µl, yielding final concentrations of 2 µM DOP Primer (5'-CCG ACT CGA GNN NNN NAT

30  GTG G-3' (SEQ ID NO:20); N = A, G, C, or T in approximately equal proportions), 200 µM dNTPs, 10 mM Tris Cl (pH 8.3), 0.005% (v/v) BRIJ 35, 1.5 mM MgCl$_2$, and 50 mM KCl. Radioactively labeled α-[$^{32}$P] dCTP, approximately 60 cpm/pmol total dNTPs was added to the reaction for quantitation of DNA synthesis as described for MDA product

quantitation in Example 6. After the initial denaturation of the template at 95°C for 5 min, 2.5 units Taq DNA Polymerase (Invitrogen Life Technologies, Carlsbad, CA) was added, followed by 5 cycles of 94°C for 1 min, 30°C for 1.5 min, ramping up to 72°C in 3 min and elongation at 72°C for 3 min, and then 35 cycles of 94°C for 1 min, 62°C for 1 min, and

5   72°C for 2 min (+14 extra seconds/ cycle). A final elongation was done at 72°C for 7 min. Amplification reactions were carried out using a GeneAmp 9700 PCR Systems Thermocycler (Applied Biosystems, Foster City, CA).

Amplification of human genomic DNA by primer extension preamplification (PEP; Zhang et al., Proc Natl Acad Sci U S A. 89:5847-5851 (1992)) was carried out as follows.

10   Human genomic DNA (ranging from 300ng to 0.03ng) was placed into 0.2 ml tubes in a total volume of 60 µl, yielding final concentrations of 33uM PEP random primer (5'-NNN NNN NNN NNN NNN-3'), 100uM dNTPs, 10 mM Tris-HCl, pH 8.3 (20°C), 1.5 mM MgCl$_2$, 50 mM KCl, and 5 units of Taq DNA Polymerase (Invitrogen Life Technologies, Carlsbad, CA). Radioactively labeled $\alpha$-[$^{32}$P] dCTP, approximately 60 cpm/pmol total

15   dNTPs was added to the reaction for quantitation of PCR product yield as described for MDA product quantitation in Example 6. The PEP reaction was performed for 50 cycles at 92°C for 1 min, 37°C for 2 min, ramping up to 55°C at 10 sec/degree, and elongation at 55°C for 4 min. PEP reactions were carried out using a GeneAmp 9700 PCR Systems Thermocycler (Applied Biosystems, Foster City, CA).

20   TaqMan® quantitative PCR analysis was performed as described in Example 8, and maximum amplification bias between loci was calculated by dividing the high locus representation value by the low value for each level of fold amplification. The results are shown in Figure 18.

The relative representation of eight loci is depicted in Figure 18 for amplification

25   reactions carried out by three different WGA procedures. The X-axis represents the fold amplification in the amplified DNA used as template for quantitative PCR; the Y-axis is the locus representation, expressed as a percent, relative to input genomic DNA (such a locus representation can be referred to as a locus representation bias), which is calculated as the yield of quantitative PCR product from 1 µg amplified DNA divided by the yield from 1 µg

30   genomic DNA control. The results for eight loci are indicated as follows; CXCR5, open diamonds; connexin40, open triangles; MKP1, open squares; CCR6, open circles; acidic ribosomal protein, filled diamonds; CCR1, filled triangles; cJUN, filled squares; CCR7, filled circles. Figure 18A depicts the percent representation for eight loci derived from

MDA-amplified DNA. Figure 18B depicts the percent representation for eight loci present in DNA amplified using DOP-PCR. Figure 18C depicts the percent representation for eight loci present in PEP-amplified DNA.

5    As can be seen, for 100-, 1,000-, and 10,000-fold amplified MDA, the maximum amplification biases were only 2.7, 2.3, and 2.8 respectively, expressed as the ratio of the most highly represented gene to the least represented gene. Significantly, the 3-fold amplification bias of MDA remained almost constant between 100- and 100,000-fold amplification (Figure 18A). In contrast, amplification by the DOP-PCR method exhibited an amplification bias ranging between 4 and 6 orders of magnitude (Figure 18B). In addition,

10   the PEP method exhibited an amplification bias spanning 2-4 orders of magnitude (Figure 18C).

**J.   Example 10:  Amplification using nested primers yields specific amplification of c-jun sequences.**

This example demonstrates the amplification of a specific DNA region from a

15   complex mixture of DNA sequences using $\phi$29 DNA polymerase with sequence-specific, nested primers.

Amplification reactions were carried out either with or without a heat denaturing/annealing step using either exonuclease-resistant hexamers or a nested set of 19 exonuclease-resistant sequence-specific primers. The nested primers used in this example

20   are listed in Table 5. The presence of an asterisk in the nucleotide sequence indicates the presence of a phosphorothioate bond. The nested primers are designed to hybridize to opposite strands on each side of the human c-jun gene, and the closest left and right primers encompass a 3420 bp fragment containing the c-jun gene. On each side of the c-jun gene, these nested primers are spaced 150-400 nucleotides between each other. The region of

25   human DNA encompassing the recognition sites for these primers can be accessed from Genbank using Accession Number AL136985 and spans positions 65001 to 73010 of the nucleotide sequence.

**Table 5**

Left Primers (5' to 3')

| c-Jun L9 | TCC ATC ACG  AGT TAT GC*A* C | (SEQ ID NO:1) |
|----------|------------------------------|---------------|
| c-Jun L8 | TGG AGT TAC TAA GGG AA*G* C  | (SEQ ID NO:2) |
| c-Jun L7 | ACT GAG TTC ATG AAC CC*T* C  | (SEQ ID NO:3) |
| c-Jun L6 | ATT AAC TCA TTG AAG GC*C* C  | (SEQ ID NO:4) |

| c-Jun L5 | TCT GTG CTG TAC TGT TG*T* C | (SEQ ID NO:5) |
| c-Jun L4 | AGT TTG GCA AAC TGG GC*T* C | (SEQ ID NO:6) |
| c-Jun L3 | TGG CTC TTG GTA TGA AA*A* G | (SEQ ID NO:7) |
| c-Jun L2 | ACT GTT AGT TTC CAT AG*G* C | (SEQ ID NO:8) |
| c-Jun L1 | TGA ATA CAT TTA TTG TG*A* C | (SEQ ID NO:9) |

Right Primers (5' to 3')

| c-Jun R1 | CGA CTG TAG GAG GGC AG*C* G | (SEQ ID NO:10) |
| c-Jun R2 | CGT CAG CCC ACA ATG CA*C* C | (SEQ ID NO:11) |
| c-Jun R3 | GTA CTT GGA TTC TCA GC*C* T | (SEQ ID NO:12) |
| c-Jun R4 | CAA ATC TCT CGG CTT CT*A* C | (SEQ ID NO:13) |
| c-Jun R5 | CGT GTT GTG TTA AGC GT*G* T | (SEQ ID NO:14) |
| c-Jun R6 | CCG CGG AAA AGG AAC CA*C* T | (SEQ ID NO:15) |
| c-Jun R7 | CTC CTG GCA GCC CAG TG*A* G | (SEQ ID NO:16) |
| c-Jun R8 | CTC CTC CCC TCG ATG CT*T* C | (SEQ ID NO:17) |
| c-Jun R9 | CAG TTA CCC TCT GCA GA*T* C | (SEQ ID NO:18) |
| c-Jun R10 | CTA TTT CCT CTG CAG AT*A* A | (SEQ ID NO:19) |

Four reactions were carried out under the following conditions. Human genomic DNA (50 ng) was placed into a 0.2 ml tube in a total volume of 50 μl, yielding final concentrations of 25 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM MgCl$_2$, and 100 μM exonuclease-resistant hexamer or 1 μM each of exonuclease-resistant nested primers. A heat-treatment step to increase primer annealing was included or omitted, as indicated, for individual reactions. Annealing reactions were heated to 95°C for 3 minutes and slowly cooled down to 37°C in a PCR System Thermocycler (Perkin Elmer). Reactions were divided into two, each had 25 μl and then was brought to a final volume of 50 μl, containing final concentrations of 37 mM Tris-HCl, pH 8.0, 50 mM KCl, 10 mM MgCl$_2$, 5 mM (NH$_4$)$_2$SO$_4$, 1.0 mM dNTPs, 1 unit/ml of yeast pyrophosphatase, 50 μM exonuclease-resistant hexamer or 0.5 μM exonuclease-resistant nested primers, and 800 units/ml φ29 DNA polymerase. Radioactively labeled α-[$^{32}$P] dCTP, approximately 60 cpm/pmol total dNTPs, was added to one of two parallel reactions for quantification of DNA synthesis. Reactions were incubated for 18 hours at 37°C. Incorporation of acid-precipitable

radioactive deoxyribonucleotide product was determined with glass fiber filters. The reactions that did not contain α-[$^{32}$P] dCTP were analyzed by TaqMan® quantitative PCR analysis as described in Example 8. A standard curve for input template was generated to determine the locus copy number of the amplified DNA sample relative to that of genomic

5      DNA. The standard curve was generated from 0, 0.001, 0.01, 0.1, 0.5, and 1 μg of genomic DNA. The results are shown in Figure 19.

The Y-axis is the locus representation relative to input genomic DNA (such a locus representation can be referred to as a locus representation bias). It is calculated as the yield of quantitative PCR product from 1 μg amplified DNA divided by the yield from 1 μg

10     genomic DNA control, expressed as a percent.

As can be seen, the representation of c-jun sequences amplified with random hexamers from DNA heated to 95°C was 69% (see RH (heat) bar). The representation of c-jun sequences amplified with nested primers from DNA heated to 95°C was only 3% (see NP (heat) bar). The representation of c-jun sequences in DNA amplified with random

15     hexamers without template DNA heat treatment was 211% (see RH (no heat) bar). The representation of c-jun sequences in DNA amplified with nested primers without template DNA heat treatment was 2828% (see NP (no heat) bar).

## K. Example 11: Amplification Bias of Loci Amplified by MDA from Whole Blood

This example demonstrates that genomic DNA can be amplified using MDA directly

20     from whole blood or from tissue culture cells and that the locus representation is substantially the same as for DNA amplified from purified genomic DNA template. This example illustrates lysis by subjecting cells to alkaline conditions (by addition of a lysis solution) without any lysis by heating, stabilization of the cell lysate (by addition of a stabilization solution), and multiple displacement amplification of genomic DNA in the

25     stabilized cell lysate. The stabilized cell lysate is used for amplification without purification of the genomic DNA. As a control, DNA amplified in the absence of added template was tested and contains no detectable sequence representation for these loci.

DNA was prepared from blood or a tissue culture cell line as follows. Human blood samples were obtained from Grove Hill Laboratory. U266, a myeloma cell line, was

30     obtained from ATCC and passaged according to the accompanying protocol. Cells were lysed in an alkaline lysis solution by a modification of Zhang et al. (Zhang, L. *et al.* Whole genome amplification from a single cell: implications for genetic analysis. *Proc Natl Acad Sci U S A.* 89, 5847-5851 (1992)). Blood was diluted 3-fold in PBS (137 mM NaCl, 2.7

mM KCl, 9.5 mM Na, KPO$_4$, pH 7.4), while tissue culture cells were diluted to 30,000 cells/ml in PBS. Blood or cells were lysed by dilution with an equal volume of Alkaline Lysis Buffer (400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA) and incubated 10 min on ice. The lysed cells were neutralized with the same volume of Neutralization Buffer

5     (400 mM HCl, 600 mM Tris-HCl, pH 7.5). Preparations of lysed blood or cells (1 µl) were used directly as template in MDA reactions (100 µl) as described.

MDA reactions (100 µl) were carried out without denaturation at 95°C as described in Example 8. Reactions using purified human genomic DNA template contained 300 or 30 ng DNA, resulting in approximately 100- or 1000-fold DNA amplification. Reactions using

10    DNA from lysed blood or cells contained 1 µl of the stabilized cell lysates as template. Control amplification reactions contained no added template DNA.

TaqMan® quantitative PCR analysis was performed on amplified DNA samples as described in Example 8, and the results are shown in Figure 20. The relative representation of eight loci for DNA from five different amplification reactions is depicted in Figure 20.

15    The Y-axis is the locus representation, expressed as a percent, relative to input genomic DNA (such a locus representation can be referred to as a locus representation bias), which is calculated as the yield of quantitative PCR product from 1 µg of amplified DNA divided by the yield from 1 µg of genomic DNA control. Bars with declining diagonals depict the locus representation for DNA amplified from whole blood. Solid gray bars depict the locus

20    representation for DNA amplified from 30 ng purified genomic DNA (9,000 genome copies). Solid white bars depict the locus representation for DNA amplified from 300 ng purified genomic DNA (90,000 genome copies). Bars with rising diagonals depict the locus representation for DNA amplified from tissue culture cells (10 cell equivalents of DNA). Solid black bars depict the locus representation for DNA amplified from reactions with no

25    added template (the values for the data represented by the black bars are so small that the bars are not visible on the graph).

As can be seen, DNA amplified directly from whole blood or from tissue culture cells without purification has substantially the same values for locus representation as DNA amplified from purified genomic DNA template.

**L. Example 12: The Amplification Bias of Loci Amplified by MDA in Reactions Containing AAdUTP is the Same as the Amplification Bias of DNA Amplified in Reactions Containing 100% dTTP.**

This example demonstrates that genomic DNA can be amplified using MDA in

5    reactions containing AAdUTP (5-(3-aminoallyl)-2'-deoxyuridine 5'-triphosphate, Sigma-Aldrich Co.) and that the locus representation is substantially the same as for DNA amplified in reactions containing only dTTP.

MDA reactions (100 μl) containing 100% dTTP were carried out without denaturation at 95°C as described in Example 8. Reactions containing 70% AAdUTP were

10   carried out under the same conditions as reactions containing 100% dTTP, with the exception that they contained 0.7 mM AAdUTP and 0.3 mM dTTP, instead of the standard 1.0 mM dTTP. Reactions contained 1 ng human genomic DNA template, resulting in approximately 30,000-fold DNA amplification.

TaqMan® quantitative PCR analysis was performed on amplified DNA samples as

15   described in Example 8, and the relative representation of eight loci for DNA from two amplification reactions is depicted in Figure 21. The Y-axis is the locus representation, expressed as a percent, relative to input genomic DNA (such a locus representation can be referred to as a locus representation bias), which is calculated as the yield of quantitative PCR product from 1 μg of amplified DNA divided by the yield from 1 μg of genomic DNA

20   control. Black bars depict the locus representation for DNA amplified in a reaction containing 100% dTTP. White bars depict the locus representation for DNA amplified in a reaction containing 30% dTTP / 70% AAdUTP.

As can be seen, DNA amplified in reactions containing 30% dTTP / 70% AAdUTP has substantially the same values for locus representation as DNA amplified in reactions

25   containing 100% dTTP.

**M. Example 13: Amplification of c-jun sequences from human genomic DNA using sequence specific primers and target circularization.**

This example describes an embodiment of the disclosed method and analysis of the resulting DNA products. The exemplified method is the disclosed gene-specific multiple

30   displacement amplification form of multiple strand displacement amplification using nuclease-resistant sequence-specific primers and circularized DNA template.

Amplification reactions were carried out without a heat denaturing/annealing step under the conditions described in Example 8 using exonuclease-resistant sequence-specific

primers that hybridize to sequences within a 5.5kb EcoRI fragment that contains the c-jun sequence. The sequence-specific primers used in this example are listed in Table 6. An asterisk in the nucleotide sequence indicates the presence of a phosphorothioate bond. The sequence-specific primers are designed to hybridize to opposite strands on each side of the c-jun gene sequence, and the primers encompass a 2025 bp fragment containing the c-jun gene sequence. The primers are spaced 150-400 nucleotides between each other on each side of the c-jun gene sequence. The region of human DNA encompassing the recognition sites for these primers can be accessed from Genbank using Accession Number AL136985 and spans positions 66962 to 68987 of the nucleotide sequence.

**Table 6**

Left Primers (5' to 3')

| c-Jun D1 | CTG AAA CAT CGC ACT AT*C *C | (SEQ ID NO:21) |
|---|---|---|
| c-Jun D2 | CCA AAC TTT GAA ATG TT*T *G | (SEQ ID NO:22) |
| c-Jun D3 | CTG CCA CCA ATT CCT GC*T *T | (SEQ ID NO:23) |
| c-Jun D4 | CAT AAG CAA AGG CCA TC*T *T | (SEQ ID NO:24) |
| c-Jun D5 | GGA AGC AAT TCA AGA TC*T *G | (SEQ ID NO:25) |
| c-Jun D6 | CTT CAG ATT GCA GCA AT*G *T | (SEQ ID NO:26) |
| c-Jun D7 | GAA TTA ATG AAA TTG GG*A *G | (SEQ ID NO:27) |
| c-Jun D8 | ACT GTT AGT TTC CAT AG*G *C | (SEQ ID NO:28) |
| c-Jun D9 | CAA GGT TGA TTA TTT TA*G *A | (SEQ ID NO:29) |
| c-Jun D10 | AGT ACT AGT TCA TGT TT*T *C | (SEQ ID NO:30) |

Right Primers (5' to 3')

| c-Jun U1 | TAG TAC TCC TTA AGA AC*A *C | (SEQ ID NO:31) |
|---|---|---|
| c-Jun U2 | CTA ACA TTC GAT CTC AT*T *C | (SEQ ID NO:32) |
| c-Jun U3 | GCG GAC GGG CTG TCC CC*G *C | (SEQ ID NO:33) |
| c-Jun U4 | GGA AGG ACT TGG CGC GC*C *C | (SEQ ID NO:34) |
| c-Jun U5 | AAC TAA AGC CAA GGG TA*T *C | (SEQ ID NO:35) |
| c-Jun U6 | ATA ACA CAG AGA GAC A*G *A | (SEQ ID NO:36) |
| c-Jun U7 | CAA CTC ATG CTA ACG CA*G *C | (SEQ ID NO:37) |
| c-Jun U8 | GGA AGC TGG AGA GAA TC*G *C | (SEQ ID NO:38) |

| c-Jun U9 | GAC ATG GAG TCC CAG GA*G *C | (SEQ ID NO:39) |
| c-Jun U10 | AGG CCC TGA AGG AGG AG*C *C | (SEQ ID NO:40) |

Four reactions were carried out under the following conditions. Human genomic DNA (5 μg, Coriell Cell Repositories) was digested with 100 units of EcoRI for 3 hours in 50 μl according to the manufacturers conditions. The EcoRI endonuclease was inactivated

5    by incubation at 65°C for 30 min. Digested DNA fragments (0.5 μg) were circularized in an 840 μl volume using 1.7 units (Weiss units) of T4 DNA ligase. The reaction was incubated for 16 h at 4°C. A mock ligation was carried out under identical conditions except for the omission of DNA ligase. Two amplification reactions utilizing a portion of the ligated mixture as template (16.8 μl; 10 ng DNA) were carried out, one with sequence-specific

10   primers at a concentration of 2.5 μM each and the other with the primers at a concentration of 0.5 μM each. Two more amplification reactions were carried out utilizing the mock-ligated DNA as template with sequence-specific primer concentrations of 2.5 μM and 0.5 μM. Radioactively labeled α-[$^{32}$P] dCTP, approximately 60 cpm/pmol total dNTPs, was added to parallel reactions for quantification of DNA synthesis. Reactions were incubated

15   for 18 hours at 37°C. The reactions that did not contain α-[$^{32}$P] dCTP were analyzed by TaqMan® quantitative PCR analysis as described in Example 8. A standard curve for input template was generated to determine the locus copy number of the amplified DNA sample relative to that of genomic DNA. The standard curve was generated from 0, 0.001, 0.01, 0.1, 0.5, and 1 μg of genomic DNA. The results are shown in Figure 22. The Y-axis is the

20   locus representation relative to input genomic DNA (such a locus representation can be referred to as a locus representation bias). It is calculated as the yield of quantitative PCR product from 1 μg amplified DNA divided by the yield from 1 μg genomic DNA control, expressed as a percent.

As can be seen, the representation of c-jun sequences amplified with 2.5 μM Gene-

25   Specific primers from ligated DNA was below detection (see GS 2.5 +L bar). The representation of c-jun sequences amplified with 2.5 μM Gene-Specific primers from mock-ligated DNA was also not detected (see GS 2.5 -L bar). The representation of c-jun sequences in DNA amplified with 0.5 μM Gene-Specific primers from ligated DNA was 32000% (see GS 0.5 +L bar). The representation of c-jun sequences in DNA amplified with

30   0.5 μM Gene-Specific primers from mock-ligated DNA was also not detected (see GS 0.5 -

L bar). These results demonstrate a 320-fold amplification of the c-jun sequences using gene-specific primers and circularized template DNA. Digested DNA that was not ligated did not show any appreciable amplification. Only ligated DNA that was amplified with 0.5 μM primers was amplified, while 2.5 μM primers did not yield any amplification.

5          The specificity of the DNA amplification reaction carried out with 2.5 μM Gene-Specific primers and circularized DNA template was tested by comparing it to the amount of DNA amplification observed at seven other loci. TaqMan® quantitative PCR analysis was performed as described in Example 8, and the results are shown in Figure 23. The relative representation of the c-jun locus and seven other loci is depicted and the seven loci

10        showed only low levels of amplification, indicating that the c-jun sequences were specifically amplified using the c-jun specific primers.

**N. Example 14: Increasing the quality of Multiple Displacement Amplification of degraded genomic DNA by repairing the damaged sample.**

This example demonstrates that the quality of amplified DNA in WGA is increased

15        when the damaged genomic DNA is pretreated with a repair method by denatured with 70°C heat in the presence of 15 mM NaOH, adding Tris-HCl pH 4.0 and original sample back to the denatured sample, then slow cooling to hybridize the damaged DNA. The damaged gDNA was created by sonicating intact genomic DNA to an average length of 1 kb. This degraded sample was then pretreated with the repair method and placed into a

20        multiple displacement amplification reaction. The quality of the amplification products were assessed by a Taqman assay which compares the representation of a particular locus on a chromosome of 1 μg of amplification product to a 1 μg standard genomic DNA that has not been amplified. This example demonstrates that damaged genomic DNA with the repair treatment has 3-fold improvement in locus representation of the amplified products

25        compared to amplified products of damaged genomic DNA without the repair method.

The damaged genomic DNA was created as follows: Promega's genomic DNA sample was diluted to 100 ngs/μl in dH$_2$0. The sample was sonicated with a 550 Sonic Demembrator by Fisher Scientific setting 40% for 9 seconds. The sample was diluted further to 5 ng/μl and 25 ngs total was used to denature with 15 mM NaOH final and heated

30        to 70°C for 5 minutes. At 70°C, Tris-HCl pH 4.0 was added to create a final pH of 8.0 and 25 ng of native original denatured samples was also added. Amplification by multiple displacement synthesis was performed on repaired damaged DNA and damaged DNA, and a Control intact DNA. The results in Figure 24 show that the repair of the damaged DNA

has significantly improved the amplification of the damaged sample compared to amplification of damaged DNA without repair.

### O. Example 15: Increasing the quality of Multiple Displacement Amplification of real genomic DNA samples damaged by prolonged storage by repairing the damaged sample.

This example demonstrates that the quality of amplified product by multiple displacement amplification of genomic DNA samples damaged by prolong storage was improved by the repair method.

The damaged genomic DNA samples were pretreated with a repair method by denaturing with 70°C heat in the presence of 15 mM NaOH, adding Tris-HCl pH 4.0 and original sample back to the denatured sample, slow cooling to hybridize the damaged DNA, then placing into a multiple displacement amplification reaction. The quality of the amplification products were assessed by a Taqman assay as described in Example 14.

As seen in Figure 25, the quality of the amplification products of the damaged genomic DNA samples once repaired is significantly improved. On average, a 3-fold improvement in the quality of the amplification products was observed.

### P. Example 16: Optimization of the denaturation step: ionic strength, heat and NaOH is results in improved repair.

This example demonstrates that ionic strength, heat and NaOH are useful for the denaturation step in the disclosed method of repairing and amplifying damaged DNA. Damaged DNA samples were prepared as follows: DNA samples was diluted in $dH_2O$ to 5 ng/$\mu$l and were heated to 70°C for 5 minutes which degrades the samples into smaller fragments. The heated DNA samples were slow cooled and multiple displacement amplification was performed on these samples. A Taqman analysis of the amplification products from the heated DNA substrate showed that use of an ionic solution led to further degradation of the DNA, ultimately leading to poor amplification of these samples.

To determine if heating the damaged DNA in the presence of salt can improve the amplification of this substrate, damaged DNA (25 ngs) was heated to 70°C in the presence of a salt (Tris-EDTA (10 mM Tris pH 8.0 and 1 mM EDTA)) for 3 minutes, 25 ngs of native damaged sample was added back, and then the sample was cooled. This method showed some improvement on the repair of the damaged DNA. When 25 mM final of NaOH was added to the reaction before the heat step, this improved the repair method giving consistent repair of the damaged DNA. This was done by diluting damaged DNA in

Tris-EDTA (TE) to 5 ng/$\mu$l, 25 ngs of DNA was added to NaOH final concentration 25 mM, the sample was heated to 70°C for 3 minutes, Tris-HCl pH 4.0 was added to reduce the pH to 8.0, 25 ngs of the original native damaged DNA sample was added back, and the mixture was cooled to room temperature. Repair with heat, Tris, and NaOH gave better

5      locus representation than the no repair and repair with heat and Tris only treatments.

Q.   **Example 17: Neutralization step of the repair method improves amplification of damaged DNA sample.**

This example demonstrates that the neutralization of the repair method is important for improved quality of amplification products. A damaged DNA sample was prepared as

10     follows: a DNA sample was diluted in dH$_2$O to 10 ng/$\mu$l and was heated to 70°C for 5 minutes to create damaged DNA samples. The heated DNA sample was diluted to 5 ngs/$\mu$l in TE, 25 ngs of damaged DNA was added to final 25 mM NaOH, heated to 70°C for 3 minutes and was either neutralized with Tris-HCl pH 4.0 to a pH of 8.0 and slow cooled or was slow cooled without neutralization. Amplification reactions of these samples were

15     performed and the quality of products were analyzed by Taqman analysis. Repair with neutralization gave a better locus representation than the no repair and repair without neutralization treatments.

It is understood that the disclosed invention is not limited to the particular methodology, protocols, and reagents described as these may vary. It is also to be

20     understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a ", "an", and "the" include plural reference unless the context clearly dictates otherwise.

25     Thus, for example, reference to "a host cell" includes a plurality of such host cells, reference to "the antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed

30     invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are as described. Publications cited herein and the material for which they are cited are specifically incorporated by reference. Nothing herein is to be

construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

## CLAIMS

We claim:

1. A method of amplifying genomes, the method comprising,

bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample,

wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the genomic nucleic acid sample.

2. The method of claim 1 wherein the genome is a eukaryotic genome.

3. The method of claim 2 wherein the genome is a plant genome.

4. The method of claim 2 wherein the genome is an animal genome.

5. The method of claim 4 wherein the genome is a vertebrate genome.

6. The method of claim 5 wherein the genome is a fish genome.

7. The method of claim 5 wherein the genome is a mammalian genome.

8. The method of claim 7 wherein the genome is a human genome.

9. The method of claim 1 wherein the genome is a microbial genome or a viral genome.

10. The method of claim 1 wherein the amplification bias is less than 20-fold for at least ten nucleic acid sequences in the genomic nucleic acid sample.

11. The method of claim 10 wherein the amplification bias is less than 10-fold for at least ten nucleic acid sequences in the genomic nucleic acid sample.

12. The method of claim 1 wherein the primer has a length of 3 nucleotides, 4 nucleotides, 5 nucleotides, 6 nucleotides, 7 nucleotides, 8 nucleotides, 9 nucleotides, 10 nucleotides, 11 nucleotides, 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides.

13. The method of claim 1 wherein the primer has a length of less than 4 nucleotides, less than 5 nucleotides, less than 6 nucleotides, less than 7 nucleotides, less than 8

nucleotides, less than 9 nucleotides, less than 10 nucleotides, less than 11 nucleotides, less than 12 nucleotides, less than 13 nucleotides, less than 14 nucleotides, less than 15 nucleotides, less than 16 nucleotides, less than 17 nucleotides, less than 18 nucleotides, less than 19 nucleotides, less than 20 nucleotides, less than 21 nucleotides, less than 22 nucleotides, less than 23 nucleotides, less than 24 nucleotides, less than 25 nucleotides, less than 26 nucleotides, less than 27 nucleotides, less than 28 nucleotides, less than 29 nucleotides, less than 30 nucleotides, or less than 31 nucleotides.

14. The method of claim 1 wherein the genomic nucleic acid sample is incubated at 20°C, 21°C, 22°C, 23°C, 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, 34°C, 35°C, 36°C, 37°C, 38°C, 39°C, 40°C, 41°C, 42°C, 43°C, 44°C, 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 67°C, 68°C, 69°C, 70°C, 71°C, 72°C, 73°C, 74°C, 75°C, 76°C, 77°C, 78°C, 79°C, or 80°C.

15. The method of claim 1 wherein the genomic nucleic acid sample is incubated at less than 21°C, less than 22°C, less than 23°C, less than 24°C, less than 25°C, less than 26°C, less than 27°C, less than 28°C, less than 29°C, less than 30°C, less than 31°C, less than 32°C, less than 33°C, less than 34°C, less than 35°C, less than 36°C, less than 37°C, less than 38°C, less than 39°C, less than 40°C, less than 41°C, less than 42°C, less than 43°C, less than 44°C, less than 45°C, less than 46°C, less than 47°C, less than 48°C, less than 49°C, less than 50°C, less than 51°C, less than 52°C, less than 53°C, less than 54°C, less than 55°C, less than 56°C, less than 57°C, less than 58°C, less than 59°C, less than 60°C, less than 61°C, less than 62°C, less than 63°C, less than 64°C, less than 65°C, less than 66°C, less than 67°C, less than 68°C, less than 69°C, less than 70°C, less than 71°C, less than 72°C, less than 73°C, less than 74°C, less than 75°C, less than 76°C, less than 77°C, less than 78°C, less than 79°C, or less than 80°C.

16. The method of claim 1 wherein the genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^3$ nucleotides, the genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^4$ nucleotides, the genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^5$ nucleotides, the genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^6$ nucleotides, the genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^7$ nucleotides, the genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^8$ nucleotides, or the genomic nucleic acid sample has a sequence complexity of at least $1 \times 10^9$ nucleotides.

17. The method of claim 1 wherein the primer, DNA polymerase and genomic nucleic acid sample are brought into contact with 1 additional primer, with 2 additional primers, with 3 additional primers, with 4 additional primers, with 5 additional primers, with 6 additional primers, with 7 additional primers, with 8 additional primers, with 9 additional primers, with 10 additional primers, with 11 additional primers, with 12 additional primers, with 13 additional primers, with 14 additional primers, with 15 additional primers, with 16 additional primers, with 17 additional primers, with 18 additional primers, with 19 additional primers, with 20 additional primers, with 21 additional primers, with 22 additional primers, with 23 additional primers, with 24 additional primers, with 25 additional primers, with 26 additional primers, with 27 additional primers, with 28 additional primers, with 29 additional primers, with 30 additional primers, with 31 additional primers, with 32 additional primers, with 33 additional primers, with 34 additional primers, with 35 additional primers, with 36 additional primers, with 37 additional primers, with 38 additional primers, with 39 additional primers, with 40 additional primers, with 41 additional primers, with 42 additional primers, with 43 additional primers, with 44 additional primers, with 45 additional primers, with 46 additional primers, with 47 additional primers, with 48 additional primers, with 49 additional primers, with 50 additional primers, with 51 additional primers, with 52 additional primers, with 53 additional primers, with 54 additional primers, with 55 additional primers, with 56 additional primers, with 57 additional primers, with 58 additional primers, with 59 additional primers, with 60 additional primers, with 61 additional primers, with 62 additional primers, with 63 additional primers, with 75 additional primers, with 100 additional primers, with 150 additional primers, with 200 additional primers, with 300 additional primers, with 400 additional primers, with 500 additional primers, with 750 additional primers, or with 1,000 additional primers, wherein each primer has a different specific nucleotide sequence.

18. The method of claim 17 wherein the primers are all of the same length.

19. The method of claim 1 wherein the primer, DNA polymerase and genomic nucleic acid sample are brought into contact with fewer than 2 additional primers, with fewer than 3 additional primers, with fewer than 4 additional primers, with fewer than 5 additional primers, with fewer than 6 additional primers, with fewer than 7 additional primers, with fewer than 8 additional primers, with fewer than 9 additional primers, with fewer than 10 additional primers, with fewer than 11 additional primers, with fewer than 12 additional primers, with fewer than 13 additional primers, with fewer than 14 additional primers, with fewer than 15 additional primers, with fewer than 16 additional primers, with fewer than 17

additional primers, with fewer than 18 additional primers, with fewer than 19 additional primers, with fewer than 20 additional primers, with fewer than 21 additional primers, with fewer than 22 additional primers, with fewer than 23 additional primers, with fewer than 24 additional primers, with fewer than 25 additional primers, with fewer than 26 additional primers, with fewer than 27 additional primers, with fewer than 28 additional primers, with fewer than 29 additional primers, with fewer than 30 additional primers, with fewer than 31 additional primers, with fewer than 32 additional primers, with fewer than 33 additional primers, with fewer than 34 additional primers, with fewer than 35 additional primers, with fewer than 36 additional primers, with fewer than 37 additional primers, with fewer than 38 additional primers, with fewer than 39 additional primers, with fewer than 40 additional primers, with fewer than 41 additional primers, with fewer than 42 additional primers, with fewer than 43 additional primers, with fewer than 44 additional primers, with fewer than 45 additional primers, with fewer than 46 additional primers, with fewer than 47 additional primers, with fewer than 48 additional primers, with fewer than 49 additional primers, with fewer than 50 additional primers, with fewer than 51 additional primers, with fewer than 52 additional primers, with fewer than 53 additional primers, with fewer than 54 additional primers, with fewer than 55 additional primers, with fewer than 56 additional primers, with fewer than 57 additional primers, with fewer than 58 additional primers, with fewer than 59 additional primers, with fewer than 60 additional primers, with fewer than 61 additional primers, with fewer than 62 additional primers, with fewer than 63 additional primers, with fewer than 64 additional primers, with fewer than 75 additional primers, with fewer than 100 additional primers, with fewer than 150 additional primers, with fewer than 200 additional primers, with fewer than 300 additional primers, with fewer than 400 additional primers, with fewer than 500 additional primers, with fewer than 750 additional primers, or with fewer than 1,000 additional primers, wherein each primer has a different specific nucleotide sequence.

20. The method of claim 19 wherein each primer has a different one of the sequences AGTGGG or AGAGAG.

21. The method of claim 19 wherein each primer has a different one of the sequences AGCCGG, AGTAGG, or AGTTGG.

22. The method of claim 19 wherein each primer has a different one of the sequences AGGCGG, AGTGGG, AGGGAG, or AGTGAG.

23. The method of claim 19 wherein each primer has a different one of the sequences AGTGGG, AGCCAG, AGTTAG, AGTCAG, or AGACAG.

24. The method of claim 19 wherein each primer has a different one of the sequences AGAGGG, AGGCAG, AGCCAG, AGTCAG, or AGACAG.

25. The method of claim 19 wherein each primer has a different one of the sequences AGTAGG, AGGTGG, AGGCAG, AGACAG, or AGTGAG.

26. The method of claim 19 wherein each primer has a different one of the sequences AGGAGG, AGAGGG, AGGGAG, AGTCAG, or AGCGAG.

27. The method of claim 19 wherein each primer has a different one of the sequences CGGTGG, TCACGC, CGAGCG, GCGTGG, ACTCGG, AATCGC, CGGAGG, CCGAGA, GATCGC, AGAGCG, AGCGAG, or ACTCCG.

28. The method of claim 1 wherein the primer has one of the sequences AGTGGG or AGAGAG.

29. The method of claim 1 wherein the primer has one of the sequences AGCCGG, AGTAGG, or AGTTGG.

30. The method of claim 1 wherein the primer has one of the sequences AGGCGG, AGTGGG, AGGGAG, or AGTGAG.

31. The method of claim 1 wherein the primer has one of the sequences AGTGGG, AGCCAG, AGTTAG, AGTCAG, or AGACAG.

32. The method of claim 1 wherein the primer has one of the sequences AGAGGG, AGGCAG, AGCCAG, AGTCAG, or AGACAG.

33. The method of claim 1 wherein the primer has one of the sequences CGGTGG, TCACGC, CGAGCG, GCGTGG, ACTCGG, AATCGC, CGGAGG, CCGAGA, GATCGC, AGAGCG, AGCGAG, or ACTCCG.

34. The method of claim 1 wherein the primer is complementary to a sequence in a repeat sequence.

35. The method of claim 34 wherein the repeat sequence is a microsatellite sequence, a minisatellite sequence, a satellite sequence, a transposon sequence, a ribosomal RNA sequence, a short interspersed nuclear element (SINE), or a long interspersed nuclear element (LINE).

36. The method of claim 1 wherein the primer is complementary to a sequence in a functional consensus sequence.

37. The method of claim 36 wherein the functional consensus sequence is a promoter sequence, an enhancer sequence, a silencer sequence, an upstream regulatory element

sequence, a transcription termination site sequence, a transposon regulatory sequence, a ribosomal RNA regulatory sequence, or a polyadenylation site sequence.

38. The method of claim 37 wherein the functional consensus sequence is a microbial promoter sequence, a microbial enhancer sequence, a microbial silencer sequence, a microbial upstream regulatory element sequence, a microbial transcription termination site sequence, a microbial transposon regulatory sequence, a microbial ribosomal RNA regulatory sequence, or a microbial polyadenylation site sequence.

39. The method of claim 1 wherein the primer is a broad coverage primer.

40. The method of claim 39 wherein the primer is complementary to a sequence that occurs every 5,000 nucleotides or less, every 4,000 nucleotides or less, every 3,000 nucleotides or less, every 2,500 nucleotides or less, every 2,000 nucleotides or less, every 1,500 nucleotides or less, every 1,000 nucleotides or less, every 900 nucleotides or less, every 800 nucleotides or less, every 700 nucleotides or less, every 600 nucleotides or less, every 500 nucleotides or less, every 400 nucleotides or less, every 300 nucleotides or less, every 200 nucleotides or less, every 100 nucleotides or less, or every 50 nucleotides or less, on average, in the nucleic acid molecules of the genomic nucleic acid sample.

41. The method of claim 39 wherein the primer has a G+C percentage within 20%, within 15%, within 10%, within 9%, within 8%, within 7%, within 6%, within 5%, within 4%, within 3%, within 2%, or within 1% of the G+C percentage of the genomic nucleic acid sample.

42. The method of claim 39 wherein the primer produces a locus representation of at least 10% for at least 5 different loci for the type of genomic nucleic acid sample used.

43. The method of claim 42 wherein the primer produces a locus representation of at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% for at least 5 different loci for the type of genomic nucleic acid sample used.

44. The method of claim 42 wherein the primer produces a locus representation of at least 10% for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at

least 50 different loci, at least 75 different loci, or at least 100 different loci for the type of genomic nucleic acid sample used.

45. The method of claim 1 wherein the primer produces an amplification bias of less than 50-fold for the type of genomic nucleic acid sample used.

46. The method of claim 45 wherein the primer produces an amplification bias of less than 45-fold, less than 40-fold, less than 35-fold, less than 30-fold, less than 25-fold, less than 20-fold, less than 19-fold, less than 18-fold, less than 17-fold, less than 16-fold, less than 15-fold, less than 14-fold, less than 13-fold, less than 12-fold, less than 11-fold, less than 10-fold, less than 9-fold, less than 8-fold, less than 7-fold, less than 6-fold, less than 5-fold, or less than 4-fold for the type of genomic nucleic acid sample used.

47. The method of claim 45 wherein the primer produces an amplification bias of less than 50-fold for at least 5 different loci, for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci for the type of genomic nucleic acid sample used.

48. The method of claim 1 wherein the primer does not have an inter-complementary 3' end.

49. The method of claim 1 wherein the primer does not produce significant replication products in the absence of a nucleic acid sample.

50. The method of claim 1 wherein the DNA polymerase is φ29 DNA polymerase.

51. The method of claim 1 wherein the genomic nucleic acid sample is not subjected to denaturing conditions.

52. The method of claim 51 wherein the genomic nucleic acid sample is not subjected to heat denaturing conditions.

53. The method of claim 51 wherein the genomic nucleic acid sample is not subjected to alkaline denaturing conditions.

54. The method of claim 1 wherein the genomic nucleic acid sample is subjected to denaturing conditions.

55. The method of claim 54 wherein the genomic nucleic acid sample is subjected to heat denaturing conditions.

317

56. The method of claim 54 wherein the genomic nucleic acid sample is subjected to alkaline denaturing conditions.

57. The method of claim 1 wherein nucleic acids in the genomic nucleic acid sample are not separated from other material in the genomic nucleic acid sample.

58. The method of claim 1 wherein the genomic nucleic acid sample is a crude cell lysate.

59. The method of claim 1 wherein the genomic nucleic acid sample is produced by exposing cells to alkaline conditions to form a cell lysate, wherein the cell lysate comprises a whole genome, and reducing the pH of the cell lysate to form a stabilized cell lysate.

60. The method of claim 59 wherein the cells are exposed to alkaline conditions by mixing the cells with a lysis solution.

61. The method of claim 60 wherein the lysis solution comprises a base.

62. The method of claim 59 wherein the pH of the cell lysate is reduced by mixing the cell lysate with a stabilization solution.

63. The method of claim 62 wherein the stabilization solution comprises a buffer.

64. The method of claim 62 wherein the stabilization solution comprises an acid.

65. The method of claim 59 wherein nucleic acids in the cell lysate and the stabilized cell lysate are not separated from other material in the cell lysate.

66. The method of claim 59 wherein the cell lysate and the stabilized cell lysate are not subjected to purification prior to the incubation.

67. The method of claim 59 wherein the cell lysate, stabilized cell lysate, or both are subjected to partial purification prior to the incubation.

68. The method of claim 59 wherein the cell lysate and the stabilized cell lysate are not subjected to substantial purification prior to the incubation.

69. The method of claim 59 wherein the incubation is substantially isothermic.

70. The method of claim 69 wherein neither the cell lysate nor the stabilized cell lysate is heated substantially above the temperature of the incubation.

71. The method of claim 69 wherein neither the cell lysate nor the stabilized cell lysate is subjected to substantial heating above the temperature of the incubation.

72. The method of claim 69 wherein the cells are not heated substantially above the temperature of the incubation.

73. The method of claim 69 wherein the cells are not subjected to substantial heating above the temperature of the incubation.

74. The method of claim 69 wherein the cells are not heated substantially above the temperature at which the cells grow.

75. The method of claim 69 wherein the cells are not subjected to substantial heating above the temperature at which the cells grow.

76. The method of claim 59 wherein neither the cell lysate nor the stabilized cell lysate is heated above a temperature and for a time that would cause notable denaturation of the genome.

77. The method of claim 59 wherein neither the cell lysate nor the stabilized cell lysate is subjected to heating above a temperature and for a time that would cause notable denaturation of the genome.

78. The method of claim 59 wherein the cells are not lysed by heat.

79. The method of claim 59 wherein the cells are not heated above a temperature and for a time that would cause substantial cell lysis in the absence of the alkaline conditions.

80. The method of claim 59 wherein the cells are not subjected to heating above a temperature and for a time that would cause substantial cell lysis in the absence of the alkaline conditions.

81. The method of claim 1 further comprising, prior to bringing into contact the primer, the genomic nucleic acid sample and the DNA polymerase,

exposing the genomic nucleic acid sample to conditions that promote substantial denaturation of the nucleic acid molecules in the genomic nucleic acid sample, thereby forming a denatured genomic nucleic acid sample, and altering the conditions to conditions that do not promote substantial denaturation of nucleic acid molecules in the genomic nucleic acid sample to form a denatured genomic nucleic acid sample.

82. The method of claim 81 wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in a longer average fragment length for the replicated nucleic acid molecules than the average fragment length in the genomic nucleic acid sample.

83. The method of claim 81 wherein the genomic nucleic acid sample, the denatured genomic nucleic acid sample, or both are exposed to ionic conditions.

84. The method of claim 81 wherein the genomic nucleic acid sample is exposed to conditions that promote substantial denaturation by mixing the genomic nucleic acid sample with a denaturing solution and by heating the genomic nucleic acid sample to a temperature and for a length of time that substantially denatures the nucleic acid molecules in the genomic nucleic acid sample.

319

85.  The method of claim 1 wherein the primer contains at least one modified nucleotide such that the primer is resistant to 3'-5' exonuclease.

86.  The method of claim 1 wherein the primer is 6 nucleotides long, wherein the primer contains at least one modified nucleotide such that the primer is nuclease resistant, and wherein the DNA polymerase is φ29 DNA polymerase.

87.  The method of claim 1 wherein the conditions that promote replication of the nucleic acid molecules are substantially isothermic.

88.  The method of claim 1 wherein the conditions that promote replication of the nucleic acid molecules do not involve thermal cycling.

89.  The method of claim 1 wherein the conditions that promote replication of the nucleic acid molecules do not include thermal cycling.

90.  The method of claim 1 wherein the primer comprises nucleotides, wherein one or more of the nucleotides are ribonucleotides.

91.  The method of claim 90 wherein from about 10% to about 50% of the nucleotides are ribonucleotides.

92.  The method of claim 90 wherein about 50% or more of the nucleotides are ribonucleotides.

93.  The method of claim 90 wherein all of the nucleotides are ribonucleotides.

94.  The method of claim 1 wherein the primer comprises nucleotides, wherein one or more of the nucleotides are 2'-O-methyl ribonucleotides.

95.  The method of claim 94 wherein from about 10% to about 50% of the nucleotides are 2'-O-methyl ribonucleotides.

96.  The method of claim 94 wherein about 50% or more of the nucleotides are 2'-O-methyl ribonucleotides.

97.  The method of claim 94 wherein all of the nucleotides are 2'-O-methyl ribonucleotides.

98.  The method of claim 1 wherein the primer comprises nucleotides, wherein the nucleotides are a mixture of ribonucleotides and 2'-O-methyl ribonucleotides.

99.  The method of claim 1 wherein the primer comprises nucleotides, wherein the nucleotides are a mixture of deoxyribonucleotides and 2'-O-methyl ribonucleotides.

100.  The method of claim 1 wherein the genomic nucleic acid sample is a blood sample, a urine sample, a semen sample, a lymphatic fluid sample, a cerebrospinal fluid sample, amniotic fluid sample, a biopsy sample, a needle aspiration biopsy sample, a cancer

320

sample, a tumor sample, a tissue sample, a cell sample, a cell lysate sample, a crude cell lysate sample, a forensic sample, an archeological sample, an infection sample, a nosocomial infection sample, a production sample, a drug preparation sample, a biological molecule production sample, a protein preparation sample, a lipid preparation sample, a carbohydrate preparation sample, or a combination thereof.

101. The method of claim 1 wherein the genomic nucleic acid sample is a crude cell lysate.

102. The method of claim 1 wherein the genomic nucleic acid sample is not processed beyond cell lysis.

103. The method of claim 1 wherein the replicated nucleic acid molecules are analyzed.

104. The method of claim 103 wherein the replicated nucleic acid molecules are analyzed using one or more DNA chips.

105. The method of claim 103 wherein the replicated nucleic acid molecules are analyzed by hybridization.

106. The method of claim 103 wherein the replicated nucleic acid molecules are analyzed by nucleic acid sequencing.

107. The method of claim 103 wherein the replicated nucleic acid molecules are stored prior to, following, or both prior to and following their analysis.

108. The method of claim 1 further comprising

bringing into contact the primer, DNA polymerase, and a second genomic nucleic acid sample, and incubating the second genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the second genomic nucleic acid sample,

wherein the second genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the second genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the second genomic nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the second genomic nucleic acid sample.

109. The method of claim 108 wherein the second genomic nucleic acid sample is a sample from the same type of organism as the first genomic nucleic acid sample.

110. The method of claim 108 wherein the second genomic nucleic acid sample is a sample from the same type of tissue as the first genomic nucleic acid sample.

111. The method of claim 108 wherein the second genomic nucleic acid sample is a sample from the same organism as the first genomic nucleic acid sample.

112. The method of claim 111 wherein the second genomic nucleic acid sample is obtained at a different time than the first genomic nucleic acid sample.

113. The method of claim 108 wherein the second genomic nucleic acid sample is a sample from a different organism than the first genomic nucleic acid sample.

114. The method of claim 108 wherein the second genomic nucleic acid sample is a sample from a different type of tissue than the first genomic nucleic acid sample.

115. The method of claim 108 wherein the second genomic nucleic acid sample is a sample from a different species of organism than the first genomic nucleic acid sample.

116. The method of claim 108 wherein the second genomic nucleic acid sample is a sample from a different strain of organism than the first genomic nucleic acid sample.

117. The method of claim 108 wherein the second genomic nucleic acid sample is a sample from a different cellular compartment than the first genomic nucleic acid sample.

118. A method of amplifying genomes, the method comprising,

bringing into contact fewer than 1,000 primers, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample,

wherein each primer has a different specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the genomic nucleic acid sample.

119. The method of claim 118 wherein the DNA polymerase and genomic nucleic acid sample are brought into contact with fewer than 2 primers, with fewer than 3 primers, with fewer than 4 primers, with fewer than 5 primers, with fewer than 6 primers, with fewer than 7 primers, with fewer than 8 primers, with fewer than 9 primers, with fewer than 10 primers, with fewer than 11 primers, with fewer than 12 primers, with fewer than 13 primers, with fewer than 14 primers, with fewer than 15 primers, with fewer than 16 primers, with fewer than 17 primers, with fewer than 18 primers, with fewer than 19 primers, with fewer than 20 primers, with fewer than 21 primers, with fewer than 22 primers, with fewer than 23 primers, with fewer than 24 primers, with fewer than 25 primers, with fewer than 26 primers,

with fewer than 27 primers, with fewer than 28 primers, with fewer than 29 primers, with fewer than 30 primers, with fewer than 31 primers, with fewer than 32 primers, with fewer than 33 primers, with fewer than 34 primers, with fewer than 35 primers, with fewer than 36 primers, with fewer than 37 primers, with fewer than 38 primers, with fewer than 39 primers, with fewer than 40 primers, with fewer than 41 primers, with fewer than 42 primers, with fewer than 43 primers, with fewer than 44 primers, with fewer than 45 primers, with fewer than 46 primers, with fewer than 47 primers, with fewer than 48 primers, with fewer than 49 primers, with fewer than 50 primers, with fewer than 51 primers, with fewer than 52 primers, with fewer than 53 primers, with fewer than 54 primers, with fewer than 55 primers, with fewer than 56 primers, with fewer than 57 primers, with fewer than 58 primers, with fewer than 59 primers, with fewer than 60 primers, with fewer than 61 primers, with fewer than 62 primers, with fewer than 63 primers, with fewer than 64 primers, with fewer than 75 primers, with fewer than 100 primers, with fewer than 150 primers, with fewer than 200 primers, with fewer than 300 primers, with fewer than 400 primers, with fewer than 500 primers, with fewer than 750 primers, or with fewer than 1,000 primers.

120. A method of amplifying nucleic acid samples of notable sequence complexity, the method comprising,

bringing into contact a single primer, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under conditions that promote replication of nucleic acid molecules in the nucleic acid sample,

wherein the primer has a specific nucleotide sequence, wherein the nucleic acid sample has a sequence complexity of at least $1 \times 10^4$ nucleotides, wherein replication of nucleic acid molecules in the nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the nucleic acid sample.

121. The method of claim 120 wherein the nucleic acid sample has a sequence complexity of at least $1 \times 10^5$ nucleotides, the nucleic acid sample has a sequence complexity of at least $1 \times 10^6$ nucleotides, the nucleic acid sample has a sequence complexity of at least $1 \times 10^7$ nucleotides, the nucleic acid sample has a sequence complexity of at least $1 \times 10^8$ nucleotides, or the nucleic acid sample has a sequence complexity of at least $1 \times 10^9$ nucleotides.

122. The method of claim 120 wherein the nucleic acid sample is or is derived from a genome, a chromosome, a chromosome fragment, an artificial chromosome, a yeast artificial chromosome, a bacterial artificial chromosome, a cosmid, or a combination.

123. The method of claim 120 wherein the nucleic acid sample is or is derived from a blood sample, a urine sample, a semen sample, a lymphatic fluid sample, a cerebrospinal fluid sample, amniotic fluid sample, a biopsy sample, a needle aspiration biopsy sample, a cancer sample, a tumor sample, a tissue sample, a cell sample, a cell lysate sample, a crude cell lysate sample, a forensic sample, an archeological sample, an infection sample, a nosocomial infection sample, a production sample, a drug preparation sample, a biological molecule production sample, a protein preparation sample, a lipid preparation sample, a carbohydrate preparation sample, or a combination thereof.

124. The method of claim 120 wherein the nucleic acid sample is or is derived from a eukaryote, a plant, and animal, a marine animal, a vertebrate, a mammal, or a human.

125. A method of amplifying genomes, the method comprising,

bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample,

wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein the genomic nucleic acid sample has a sequence complexity of at least 1 X $10^9$ nucleotides, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 0.01% of the nucleic acid sequences in the genomic nucleic acid sample.

126. The method of claim 125 wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 0.1% of the nucleic acid sequences in the genomic nucleic acid sample, at least 1% of the nucleic acid sequences in the genomic nucleic acid sample, at least 5% of the nucleic acid sequences in the genomic nucleic acid sample, at least 10% of the nucleic acid sequences in the genomic nucleic acid sample, at least 20% of the nucleic acid sequences in the genomic nucleic acid sample, at least 30% of the nucleic acid sequences in the genomic nucleic acid sample, at least 40% of the nucleic acid sequences in the genomic nucleic acid sample, at least 50% of the nucleic acid sequences in the genomic nucleic acid sample, at least 60% of the nucleic acid sequences

in the genomic nucleic acid sample, at least 70% of the nucleic acid sequences in the genomic nucleic acid sample, at least 80% of the nucleic acid sequences in the genomic nucleic acid sample, at least 90% of the nucleic acid sequences in the genomic nucleic acid sample, at least 95% of the nucleic acid sequences in the genomic nucleic acid sample, at least 96% of the nucleic acid sequences in the genomic nucleic acid sample, at least 97% of the nucleic acid sequences in the genomic nucleic acid sample, at least 98% of the nucleic acid sequences in the genomic nucleic acid sample, or at least 99% of the nucleic acid sequences in the genomic nucleic acid sample.

127.  A method of amplifying genomes, the method comprising,

bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample,

wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein the genomic nucleic acid sample has a sequence complexity of at least 1 X $10^8$ nucleotides, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 0.1% of the nucleic acid sequences in the genomic nucleic acid sample.

128.  The method of claim 127 wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 1% of the nucleic acid sequences in the genomic nucleic acid sample, at least 5% of the nucleic acid sequences in the genomic nucleic acid sample, at least 10% of the nucleic acid sequences in the genomic nucleic acid sample, at least 20% of the nucleic acid sequences in the genomic nucleic acid sample, at least 30% of the nucleic acid sequences in the genomic nucleic acid sample, at least 40% of the nucleic acid sequences in the genomic nucleic acid sample, at least 50% of the nucleic acid sequences in the genomic nucleic acid sample, at least 60% of the nucleic acid sequences in the genomic nucleic acid sample, at least 70% of the nucleic acid sequences in the genomic nucleic acid sample, at least 80% of the nucleic acid sequences in the genomic nucleic acid sample, at least 90% of the nucleic acid sequences in the genomic nucleic acid sample, at least 95% of the nucleic acid sequences in the genomic nucleic acid sample, at least 96% of the nucleic acid sequences in the genomic nucleic acid sample, at least 97% of the nucleic acid sequences in the genomic nucleic acid sample, at least 98% of the nucleic

325

acid sequences in the genomic nucleic acid sample, or at least 99% of the nucleic acid sequences in the genomic nucleic acid sample.

129. A method of amplifying genomes, the method comprising,

bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample,

'     wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein the genomic nucleic acid sample has a sequence complexity of at least 1 X $10^7$ nucleotides, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 1% of the nucleic acid sequences in the genomic nucleic acid sample.

130. The method of claim 129 wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 5% of the nucleic acid sequences in the genomic nucleic acid sample, at least 10% of the nucleic acid sequences in the genomic nucleic acid sample, at least 20% of the nucleic acid sequences in the genomic nucleic acid sample, at least 30% of the nucleic acid sequences in the genomic nucleic acid sample, at least 40% of the nucleic acid sequences in the genomic nucleic acid sample, at least 50% of the nucleic acid sequences in the genomic nucleic acid sample, at least 60% of the nucleic acid sequences in the genomic nucleic acid sample, at least 70% of the nucleic acid sequences in the genomic nucleic acid sample, at least 80% of the nucleic acid sequences in the genomic nucleic acid sample, at least 90% of the nucleic acid sequences in the genomic nucleic acid sample, at least 95% of the nucleic acid sequences in the genomic nucleic acid sample, at least 96% of the nucleic acid sequences in the genomic nucleic acid sample, at least 97% of the nucleic acid sequences in the genomic nucleic acid sample, at least 98% of the nucleic acid sequences in the genomic nucleic acid sample, or at least 99% of the nucleic acid sequences in the genomic nucleic acid sample.

131. A method of amplifying genomes, the method comprising,

bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample,

wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein the genomic nucleic acid sample has a sequence complexity of at least 1 X $10^6$ nucleotides, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 10% of the nucleic acid sequences in the genomic nucleic acid sample.

132. The method of claim 131 wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 20% of the nucleic acid sequences in the genomic nucleic acid sample, at least 30% of the nucleic acid sequences in the genomic nucleic acid sample, at least 40% of the nucleic acid sequences in the genomic nucleic acid sample, at least 50% of the nucleic acid sequences in the genomic nucleic acid sample, at least 60% of the nucleic acid sequences in the genomic nucleic acid sample, at least 70% of the nucleic acid sequences in the genomic nucleic acid sample, at least 80% of the nucleic acid sequences in the genomic nucleic acid sample, at least 90% of the nucleic acid sequences in the genomic nucleic acid sample, at least 95% of the nucleic acid sequences in the genomic nucleic acid sample, at least 96% of the nucleic acid sequences in the genomic nucleic acid sample, at least 97% of the nucleic acid sequences in the genomic nucleic acid sample, at least 98% of the nucleic acid sequences in the genomic nucleic acid sample, or at least 99% of the nucleic acid sequences in the genomic nucleic acid sample.

133. A method of amplifying genomes, the method comprising,

bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample,

wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein the genomic nucleic acid sample has a sequence complexity of at least 1 X $10^5$ nucleotides, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 80% of the nucleic acid sequences in the genomic nucleic acid sample.

134. The method of claim 133 wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of at least 90% of the nucleic acid

sequences in the genomic nucleic acid sample, at least 95% of the nucleic acid sequences in the genomic nucleic acid sample, at least 96% of the nucleic acid sequences in the genomic nucleic acid sample, at least 97% of the nucleic acid sequences in the genomic nucleic acid sample, at least 98% of the nucleic acid sequences in the genomic nucleic acid sample, or at least 99% of the nucleic acid sequences in the genomic nucleic acid sample.

135. A method of amplifying genomes, the method comprising,

bringing into contact a single primer, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample,

wherein the primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in a locus representation of at least 10% for at least 5 different loci.

136. The method of claim 135 wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in a locus representation of at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% for at least 5 different loci.

137. The method of claim 135 wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in a locus representation of at least 10% for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

138. The method of claim 135 wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in an amplification bias of less than 50-fold.

139. The method of claim 138 wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in an amplification bias of less than 45-fold, less than 40-fold, less than 35-fold, less than 30-fold, less than 25-fold, less than 20-fold, less than 19-fold, less than 18-fold, less than 17-fold, less than 16-fold, less than 15-fold, less than 14-

328

fold, less than 13-fold, less than 12-fold, less than 11-fold, less than 10-fold, less than 9-fold, less than 8-fold, less than 7-fold, less than 6-fold, less than 5-fold, or less than 4-fold.

140. The method of claim 138 wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in an amplification bias of less than 50-fold for at least 5 different loci, for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

141. A method of amplifying nucleic acid samples of high sequence complexity, the method comprising,

bringing into contact a single primer, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under conditions that promote replication of nucleic acid molecules in the nucleic acid sample,

wherein the primer has a specific nucleotide sequence, wherein the nucleic acid sample has a sequence complexity of at least $1 \times 10^3$ nucleotides, wherein replication of nucleic acid molecules in the nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the nucleic acid sample results in a sequence representation of at least 10% for at least 5 different target sequences.

142. The method of claim 141 wherein replication of the nucleic acid molecules in the nucleic acid sample results in a sequence representation of at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% for at least 5 different target sequences.

143. The method of claim 141 wherein replication of the nucleic acid molecules in the nucleic acid sample results in a sequence representation of at least 10% for at least 6 different target sequences, at least 7 different target sequences, at least 8 different target sequences, at least 9 different target sequences, at least 10 different target sequences, at least 11 different target sequences, at least 12 different target sequences, at least 13 different target sequences, at least 14 different target sequences, at least 15 different target sequences, at least 16 different target sequences, at least 17 different target sequences, at least 18 different target sequences, at least 19 different target sequences, at least 20 different target sequences, at least

25 different target sequences, at least 30 different target sequences, at least 40 different target sequences, at least 50 different target sequences, at least 75 different target sequences, or at least 100 different target sequences.

144. The method of claim 141 wherein replication of the nucleic acid molecules in the nucleic acid sample results in an amplification bias of less than 50-fold.

145. The method of claim 144 wherein replication of the nucleic acid molecules in the nucleic acid sample results in an amplification bias of less than 45-fold, less than 40-fold, less than 35-fold, less than 30-fold, less than 25-fold, less than 20-fold, less than 19-fold, less than 18-fold, less than 17-fold, less than 16-fold, less than 15-fold, less than 14-fold, less than 13-fold, less than 12-fold, less than 11-fold, less than 10-fold, less than 9-fold, less than 8-fold, less than 7-fold, less than 6-fold, less than 5-fold, or less than 4-fold.

146. The method of claim 144 wherein replication of the nucleic acid molecules in the nucleic acid sample results in an amplification bias of less than 50-fold for at least 5 different target sequences, for at least 6 different target sequences, at least 7 different target sequences, at least 8 different target sequences, at least 9 different target sequences, at least 10 different target sequences, at least 11 different target sequences, at least 12 different target sequences, at least 13 different target sequences, at least 14 different target sequences, at least 15 different target sequences, at least 16 different target sequences, at least 17 different target sequences, at least 18 different target sequences, at least 19 different target sequences, at least 20 different target sequences, at least 25 different target sequences, at least 30 different target sequences, at least 40 different target sequences, at least 50 different target sequences, at least 75 different target sequences, or at least 100 different target sequences.

147. A method of amplifying genomes, the method comprising,

bringing into contact a set of primers, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample,

wherein the set of primers comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the genomic nucleic acid sample,

wherein each selected primer in the set can produce replication of at least 80% of the nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample, wherein the selection nucleic acid sample has a sequence complexity of at least $1 \times 10^8$ nucleotides.

148.  The method of claim 147 wherein the set of primers further comprises at least one additional primer.

149.  The method of claim 147 wherein the set of primers further comprises at least one non-selected primer, wherein the non-selected primer produces replication of less than 80% of the nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample.

150.  A method of amplifying genomes, the method comprising,

bringing into contact a set of primers, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample,

wherein the set of primers comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the genomic nucleic acid sample,

wherein each selected primer in the set can produce an amplification bias of less than 20-fold for at least 10 nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample, wherein the selection nucleic acid sample has a sequence complexity of at least $1 \times 10^8$ nucleotides.

151.  A method of amplifying genomes, the method comprising,

331

bringing into contact a set of primers, DNA polymerase, and a genomic nucleic acid sample, and incubating the genomic nucleic acid sample under conditions that promote replication of nucleic acid molecules in the genomic nucleic acid sample,

wherein the set of primers comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein the genomic nucleic acid sample comprises all or a substantial portion of a genome, wherein replication of nucleic acid molecules in the genomic nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the genomic nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the genomic nucleic acid sample,

wherein each selected primer in the set can produce a sequence representation of at least 10% for at least 10 nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample, wherein the selection nucleic acid sample has a sequence complexity of at least $1 \times 10^8$ nucleotides.

152. A method of amplifying nucleic acids, the method comprising,

bringing into contact a set of primers, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under conditions that promote replication of nucleic acid molecules in the nucleic acid sample,

wherein the set of primers comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein replication of nucleic acid molecules in the nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the nucleic acid sample,

wherein each selected primer in the set can produce replication of at least 80% of the nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample, wherein the selection nucleic acid sample has a sequence complexity of at least $1 \times 10^8$ nucleotides.

153. The method of claim 152 wherein the set of primers further comprises at least one additional primer.

154. The method of claim 152 wherein the set of primers further comprises at least one non-selected primer, wherein the non-selected primer produces replication of less than 80% of the nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample.

155. The method of claim 152 wherein the nucleic acid sample has a sequence complexity of at least 1 X $10^3$ nucleotides.

156. A method of amplifying nucleic acids, the method comprising,

bringing into contact a set of primers, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under conditions that promote replication of nucleic acid molecules in the nucleic acid sample,

wherein the set of primers comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein replication of nucleic acid molecules in the nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the nucleic acid sample,

wherein each selected primer in the set can produce an amplification bias of less than 20-fold for at least 10 nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample, wherein the selection nucleic acid sample has a sequence complexity of at least 1 X $10^8$ nucleotides.

157. A method of amplifying nucleic acids, the method comprising,

bringing into contact a set of primers, DNA polymerase, and a nucleic acid sample, and incubating the nucleic acid sample under conditions that promote replication of nucleic acid molecules in the nucleic acid sample,

wherein the set of primers comprises one or more selected primers, wherein each selected primer has a specific nucleotide sequence, wherein replication of nucleic acid molecules in the nucleic acid sample proceeds by strand displacement replication, wherein replication of the nucleic acid molecules in the nucleic acid sample results in replication of all or a substantial fraction of the nucleic acid molecules in the nucleic acid sample,

wherein each selected primer in the set can produce a sequence representation of at least 10% for at least 10 nucleic acid sequences in a selection nucleic acid sample when the primer, DNA polymerase, and the selection nucleic acid sample are brought into contact and incubated under conditions that promote replication of nucleic acid molecules in the selection nucleic acid sample, wherein the selection nucleic acid sample has a sequence complexity of at least 1 X $10^8$ nucleotides.

158.  A method of amplifying nucleic acids, the method comprising,

incubating nucleic acids comprising target sequences at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, and a set of primers, under conditions promoting replication of the nucleic acids, wherein replication of the nucleic acids results in replicated strands, wherein during replication at least one of the replicated nucleic acid strands is displaced by strand displacement replication of another replicated strand,

wherein formation of replicated strands from the target sequences is favored over formation of replicated strands from non-target sequences.

159.  The method of claim 158 wherein the elevated temperature is a temperature at which the nucleic acid polymerase is substantially incapable of performing template-dependent polymerization in absence of the additive.

160.  The method of claim 159 wherein the elevated temperature is a temperature greater than 30 degrees Celsius.

161.  The method of claim 159 wherein the elevated temperature is a temperature greater than 32 degrees Celsius.

162.  The method of claim 159 wherein the elevated temperature is a temperature greater than 35 degrees Celsius.

163.  The method of claim 159 wherein the elevated temperature is a temperature greater than 37 degrees Celsius.

164.  The method of claim 158 wherein the thermolabile nucleic acid polymerase is Phi29 DNA polymerase.

165.  The method of claim 158 wherein the thermolabile nucleic acid polymerase is Phi29 DNA polymerase, E.coli DNA polymerase, Bst large fragment DNA polymerase, Bca DNA polymerase, phage M2 DNA polymerase, phage φPRD1 DNA polymerase, Klenow fragment of DNA polymerase I, T5 DNA polymerase, T4 DNA polymerase holoenzyme, or a combination.

166. The method of claim 158 wherein the set of primers comprises at least 2 primers.

167. The method of claim 158 wherein the set of primers comprises at least 10 primers.

168. The method of claim 158 wherein the set of primers comprises at least 50 primers.

169. The method of claim 158 wherein the set of primers comprises greater than 200 primers.

170. The method of claim 158 wherein the set of primers comprises greater than 1023 primers.

171. The method of claim 158 wherein the primers in the set of primers are each 6 nucleotides in length.

172. The method of claim 158 wherein the primers in the set of primers are each 8 nucleotides in length.

173. The method of claim 158 wherein the primers in the set of primers are each longer than 8 nucleotides.

174. The method of claim 158 wherein two or more of the primers in the set of primers are of different lengths.

175. The method of claim 158 wherein the additive comprises a sugar or a combination of sugars.

176. The method of claim 175 wherein the additive comprises trehalose, glucose, sucrose, or a combination.

177. The method of claim 158 wherein the additive comprises a sugar, a chaperone, a protein, or a combination.

178. The method of claim 158 wherein strand displacement replication is performed in presence of the additive.

179. The method of claim 178 wherein the additive comprises trehalose.

180. The method of claim 158 wherein the incubation of the nucleic acids, nucleic acid polymerase, additive, and set of primers is in the presence of deoxyribonucleotide triphosphates.

181. The method of claim 158 wherein the ratio of replicated strands from target sequences to replicated strands from non-target sequences is less than the ratio of replicated strands from target sequences to replicated strands from non-target sequences if the nucleic

335

acids are incubated in the presence of the same nucleic acid polymerase and set of primers and under the same conditions except not at an elevated temperature.

182. The method of claim 158 wherein the elevated temperature is a temperature at or above which the nucleic acid polymerase is notably inactivated in the absence of an additive, dNTPs, and template nucleic acid.

183. The method of claim 158 wherein the elevated temperature is a temperature at or above which the nucleic acid polymerase is substantially inactivated in the absence of an additive, dNTPs, and template nucleic acid.

184. The method of claim 158 wherein the elevated temperature is a temperature at or above which the nucleic acid polymerase is significantly inactivated in the absence of an additive, dNTPs, and template nucleic acid.

185. A method of amplifying a whole genome, the method comprising,

exposing cells to alkaline conditions to form a cell lysate, wherein the cell lysate comprises a whole genome,

reducing the pH of the cell lysate to form a stabilized cell lysate, and

incubating stabilized cell lysate at an elevated temperature in the presence of a thermolabile nucleic acid polymerase having strand displacement activity, an additive, and a set of primers, under conditions promoting replication of the nucleic acids, wherein replication of the nucleic acids results in replicated strands, wherein during replication at least one of the replicated nucleic acid strands is displaced by strand displacement replication of another replicated strand,

wherein formation of replicated strands from the target sequence is favored over formation of replicated strands from non-target sequences.

186. A method of performing strand displacement nucleic acid synthesis at an elevated temperature, the method comprising,

mixing thermolabile nucleic acid polymerase having strand-displacement activity, nucleic acids comprising target sequences, a set of primers, and an additive, and

incubating at an elevated temperature and under conditions favoring hybridization of the primers to the target sequences and extension of the primers by the addition of nucleotides sequentially to the 3' end of the primer in a template-dependent manner, wherein the extension results in replication of the target sequences.

187. A kit for amplifying nucleic acids, the kit comprising

a thermolabile nucleic acid polymerase having strand displacement activity,

336

an additive, and

a set of primers,

wherein incubating nucleic acids comprising target sequences at an elevated temperature in the presence of the thermolabile nucleic acid polymerase, the additive, and the set of primers under conditions promoting replication of the nucleic acids results in replicated strands and in formation of replicated strands from the target sequences in favor of formation of replicated strands from non-target sequences.

188. The kit of claim 187 wherein the elevated temperature is a temperature at which the nucleic acid polymerase is substantially incapable of performing template-dependent polymerization in absence of the additive.

189. The kit of claim 187 wherein the thermolabile nucleic acid polymerase, the additive, and the set of primers are chosen such that incubating nucleic acids comprising target sequences at an elevated temperature in the presence of the thermolabile nucleic acid polymerase, the additive, and the set of primers under conditions promoting replication of the nucleic acids results in replicated strands and in formation of replicated strands from the target sequences in favor of formation of replicated strands from non-target sequences.

190. The kit of claim 187 wherein the nucleic acid polymerase is Phi29 DNA polymerase.

191. The kit of claim 187 wherein the additive is a sugar, a chaperone, a protein, trehalose, glucose, sucrose, or a combination.

192. The kit of claim 187 wherein the additive comprises trehalose, the set of primers comprises exonuclease-resistant random hexamer primers, and the nucleic acid polymerase comprises Phi29 DNA polymerase,

wherein the kit further comprises one or more components that, when mixed in appropriate amounts, produce a reaction mixture having final concentrations of 10 mM MgCl$_2$, 37.5 mM Tris-HCl, pH 7, 50 mM KCl, 20 mM Ammonium Sulfate, and 1 mM dNTPs.

193. The kit of claim 187 further comprising any one or a combination of a stabilization solution, a lysis solution, a reaction mix that comprises the set of primers, dithiotheitol, Phosphate-Buffered Saline, and control DNA template.

194. The kit of claim 193 wherein the stabilization solution comprises 800 mM Tris-HCl, pH 4; the lysis solution comprises 400 mM KOH, 100 mM dithiothreitol, and 10 mM EDTA; the reaction mix comprises 150 mM Tris-HCl, 200 mM KCl, 40 mM MgCl$_2$, 20 mM

337

(NH$_4$)$_2$SO$_4$, 4 mM deoxynucleotide triphosphates, and 0.2 mM random hexamer primers; the dithiothreitol is 1M dithiotheitol; and the Phosphate-Buffered Saline is 1X Phosphate-Buffered Saline, pH 7.5.

195.   A method of amplifying nucleic acids, the method comprising,

exposing a sample suspected of containing nucleic acids to alkaline conditions,

reducing the pH of all or a portion of the sample to form a stabilized sample, and

incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample,

wherein replication of the nucleic acids results in replicated strands, wherein during replication at least one of the replicated strands is displaced from nucleic acids in the sample by strand displacement replication of another replicated strand, wherein the replicated strands have low amplification bias.

196.   The method of claim 195 wherein the concentration of nucleic acids in the amplification mixture favors hybridization of primers over reassociation of the nucleic acids.

197.   The method of claim 196 wherein the concentration of nucleic acids in the amplification mixture is 10 ng/µl or less.

198.   The method of claim 196 wherein the concentration of nucleic acids in the amplification mixture is 8 ng/µl or less, 6 ng/µl or less, 5 ng/µl or less, 4 ng/µl or less, 3 ng/µl or less, 2 ng/µl or less, 1 ng/µl or less, or 0.5 ng/µl or less.

199.   The method of claim 196 wherein the concentration of nucleic acids in the amplification mixture is 100 ng/µl or less.

200.   The method of claim 195 wherein the amount of nucleic acids in the amplification mixture is at or above a threshold that can result in low amplification bias in the replicated strands.

201.   The method of claim 200 wherein the amplification mixture comprises at least 100 ng of nucleic acid.

202.   The method of claim 201 wherein the amplification sample comprises at least 150 ng, at least 200 ng, at least 300 ng, at least 400 ng, at least 500 ng, at least 1 mg, at least 2 mg, or at least 3 mg of nucleic acid.

203.   The method of claim 200 wherein the amplification mixture comprises at least 10 ng of nucleic acid.

204. The method of claim 195 wherein the amplification bias of the replicated strands is less than 20-fold for at least ten target sequences in the sample.

205. The method of claim 204 wherein the amplification bias of the replicated strands is less than 10-fold for at least ten target sequences in the sample.

206. The method of claim 195 wherein the locus representation of the replicated strands is at least 10% for at least 5 different loci.

207. The method of claim 206 wherein the locus representation of the replicated strands is at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% for at least 5 different loci.

208. The method of claim 206 wherein the locus representation of the replicated strands is at least 10% for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

209. The method of claim 195 wherein the amplification bias of the replicated strands is less than 50-fold.

210. The method of claim 209 wherein the amplification bias of the replicated strands is less than 45-fold, less than 40-fold, less than 35-fold, less than 30-fold, less than 25-fold, less than 20-fold, less than 19-fold, less than 18-fold, less than 17-fold, less than 16-fold, less than 15-fold, less than 14-fold, less than 13-fold, less than 12-fold, less than 11-fold, less than 10-fold, less than 9-fold, less than 8-fold, less than 7-fold, less than 6-fold, less than 5-fold, or less than 4-fold.

211. The method of claim 209 wherein the amplification bias of the replicated strands is less than 50-fold for at least 5 different loci, for at least 6 different loci, at least 7 different loci, at least 8 different loci, at least 9 different loci, at least 10 different loci, at least 11 different loci, at least 12 different loci, at least 13 different loci, at least 14 different loci, at least 15 different loci, at least 16 different loci, at least 17 different loci, at least 18 different loci, at least 19 different loci, at least 20 different loci, at least 25 different loci, at least 30 different loci, at least 40 different loci, at least 50 different loci, at least 75 different loci, or at least 100 different loci.

212. The method of claim 195 wherein the sample comprises cells, wherein the alkaline conditions promote lysis of the cells, wherein the alkaline conditions result in a cell lysate.

213. The method of claim 212 wherein the sample is not subjected to heating above a temperature or for a time that would cause substantial cell lysis in the absence of the alkaline conditions.

214. The method of claim 195 wherein the sample comprises nucleic acids, wherein the nucleic acids comprise a genome, wherein replication of the nucleic acids results in replication of the genome.

215. The method of claim 214 wherein replication of the nucleic acids in the sample results in replication of all or a substantial fraction of the genome.

216. The method of claim 215 wherein the genome is a eukaryotic genome, a plant genome, an animal genome, a vertebrate genome, a fish genome, a mammalian genome, a human genome, a bacterial genome, a microbial genome, or a viral genome.

217. The method of claim 214 wherein the genome comprises at least 50% of the nucleic acids in the amplification mixture.

218. The method of claim 217 wherein the genome comprises at least 90% of the nucleic acids in the amplification mixture.

219. The method of claim 217 wherein the genome comprises at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the nucleic acids in the amplification mixture.

220. The method of claim 214 wherein the nucleic acids comprise a plurality of genomes, wherein replication of the nucleic acids results in replication of a plurality of the genomes.

221. The method of claim 220 wherein at least two of the genomes are genomes of different organisms.

222. The method of claim 221 wherein at least one genome is a human genome and at least one genome is a bacterial genome, viral genome, microbial genome, or pathogen genome.

223. The method of claim 221 wherein at least one genome is a eukaryotic genome, a plant genome, an animal genome, a vertebrate genome, a fish genome, a mammalian genome, a human genome, a bacterial genome, a microbial genome, or a viral genome, and at least one

genome is a eukaryotic genome, a plant genome, an animal genome, a vertebrate genome, a fish genome, a mammalian genome, a human genome, a bacterial genome, a microbial genome, or a viral genome.

224. The method of claim 220 wherein the genomes comprise at least 50% of the nucleic acids in the amplification mixture.

225. The method of claim 224 wherein the genomes comprise at least 90% of the nucleic acids in the amplification mixture.

226. The method of claim 224 wherein the genomes comprise at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the nucleic acids in the amplification mixture.

227. The method of claim 195 wherein the sample is a eukaryotic sample, a plant sample, an animal sample, a vertebrate sample, a fish sample, a mammalian sample, a human sample, a non-human sample, a bacterial sample, a microbial sample, a viral sample, a biological sample, a serum sample, a plasma sample, a blood sample, a urine sample, a semen sample, a lymphatic fluid sample, a cerebrospinal fluid sample, amniotic fluid sample, a biopsy sample, a needle aspiration biopsy sample, a cancer sample, a tumor sample, a tissue sample, a cell sample, a cell lysate sample, a crude cell lysate sample, a tissue lysate sample, a tissue culture cell sample, a buccal swab sample, a mouthwash sample, a stool sample, a mummified tissue sample, a forensic sample, an autopsy sample, an archeological sample, an infection sample, a nosocomial infection sample, a production sample, a drug preparation sample, a biological molecule production sample, a protein preparation sample, a lipid preparation sample, a carbohydrate preparation sample, an inanimate object sample, an air sample, a soil sample, a sap sample, a metal sample, a fossil sample, an excavated material sample, a terrestrial sample, an extra-terrestrial sample, or a combination thereof.

228. The method of claim 227 wherein the sample is a serum sample or a plasma sample.

229. The method of claim 195 wherein the sample is exposed to alkaline conditions by mixing the sample with a lysis solution.

230. The method of claim 229 wherein the lysis solution comprises a base, a buffer, or a combination.

231. The method of claim 230 wherein the lysis solution comprises a base, wherein the base is potassium hydroxide.

232. The method of claim 231 wherein the lysis solution comprises 400 mM KOH.

233. The method of claim 232 wherein the lysis solution comprises 400 mM KOH and 10 mM EDTA.

234. The method of claim 231 wherein the lysis solution comprises 100 mM KOH.

235. The method of claim 234 wherein the lysis solution comprises 100 mM KOH and 2.5 mM EDTA.

236. The method of claim 229 wherein the sample is mixed with an equal volume of the lysis solution.

237. The method of claim 195 wherein the pH of the sample is reduced to the range of about pH 7.0 to about pH 6.8.

238. The method of claim 195 wherein the pH of the sample is reduced by mixing the sample with a stabilization solution.

239. The method of claim 238 wherein the stabilization solution comprises a buffer, an acid, or a combination.

240. The method of claim 239 wherein the stabilization solution comprises a buffer, wherein the buffer is Tris-HCl.

241. The method of claim 240 wherein the stabilization solution comprises 800 mM Tris-HCl.

242. The method of claim 240 wherein the stabilization solution comprises 200 mM Tris-HCl.

243. The method of claim 240 wherein the stabilization solution comprises 20 mM Tris-HCl.

244. The method of claim 238 wherein the sample is mixed with an equal volume of the stabilization solution.

245. The method of claim 195 wherein exposing the sample to alkaline conditions, reducing the pH of the sample, and incubating the stabilized sample are performed in the same reaction chamber.

246. The method of claim 245 wherein the reaction chamber comprises a tube, a test tube, an eppendorf tube, a vessel, a micro vessel, a plate, a well, a well of a micro well plate, a well of a microtitre plate, a chamber, a micro fluidics chamber, a micro machined chamber, a sealed chamber, a hole, a depression, a dimple, a dish, a surface, a membrane, a microarray, a fiber, a glass fiber, an optical fiber, a woven fiber, a film, a bead, a bottle, a chip, a compact disk, a shaped polymer, a particle, or a microparticle.

247. The method of claim 246 wherein the surface is sealable.

248. The method of claim 245 wherein the reaction chamber comprises acrylamide, cellulose, nitrocellulose, glass, gold, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, glass, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, functionalized silane, polypropylfumerate, collagen, glycosaminoglycans, polyamino acids, or a combination.

249. The method of claim 245 wherein the nucleic acids are not purified or extracted prior to incubation of the amplification mixture.

250. The method of claim 245 wherein nucleic acids in the sample are not separated from other material in the sample.

251. The method of claim 245 wherein nucleic acids in the stabilized sample are not separated from other material in the stabilized sample.

252. The method of claim 195 wherein the nucleic acids in the stabilized sample are less than 0.01% pure, less than 0.1% pure, less than 0.5% pure, less than 1% pure, less than 5% pure, less than 10% pure, or less than 20% pure by weight excluding water.

253. The method of claim 195 wherein the sample is not subjected to substantial purification prior to the incubation.

254. The method of claim 253 wherein the sample is subjected to centrifugation, extraction, chromatography, precipitation, filtration, or dialysis prior to the incubation.

255. The method of claim 195 further comprising detecting the replicated strands.

256. The method of claim 255 wherein detecting the replicated strands comprises detecting the presence, amount, or presence and amount of replicated strands.

257. The method of claim 256 wherein the presence, amount, or presence and amount of the replicated strands is accomplished by detecting the presence, amount, or presence and amount of one or more target sequences.

258. The method of claim 257 wherein the amount of a plurality of alleles, loci, or both are detected.

259. The method of claim 255 wherein detection of replicated strands indicates that the sample contains nucleic acids.

260. The method of claim 195 wherein the replicated strands are analyzed for allele dropout, wherein there is no allele dropout for 500 or more loci, 400 or more loci, 300 or more loci, 200 or more loci, 100 or more loci, 50 or more loci, 40 or more loci, 30 or more

loci, 20 or more loci, 15 or more loci, 10 or more loci, 8 or more loci, 6 or more loci, 5 or more loci, 4 or more loci, 3 or more loci, 2 or more loci, or 1 or more loci.

261. The method of claim 195 wherein the replicated strands are analyzed for allele dropout, wherein allele dropout is less than 5% for 500 or more loci, 400 or more loci, 300 or more loci, 200 or more loci, 100 or more loci, 50 or more loci, 40 or more loci, 30 or more loci, 20 or more loci, 15 or more loci, 10 or more loci, 8 or more loci, 6 or more loci, 5 or more loci, 4 or more loci, 3 or more loci, 2 or more loci, or 1 or more loci.

262. A method of detecting the presence of nucleic acids in a sample, the method comprising,

exposing the sample to alkaline conditions,

reducing the pH of the sample to form a stabilized sample, and

incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample,

wherein replication of the nucleic acids results in replicated strands,

detecting the replicated strands, wherein detection of replicated strands indicates that the sample comprises nucleic acids.

263. The method of claim 262 further comprising quantitating the replicated strands.

264. The method of claim 263 wherein the quantity of replicated strands is a measure of the amount of nucleic acids present in the sample.

265. The method of claim 262 wherein the sample comprises nucleic acids from more than two organisms, wherein detecting the replicated strands detects at least one organism.

266. The method of claim 262 wherein the sample comprises nucleic acids from a whole ecosystem, wherein detecting the replicated strands detects at least one organism.

267. The method of claim 262 wherein the sample is a substantially cell-free sample.

268. The method of claim 262 wherein the sample is a serum sample or a plasma sample.

269. The method of claim 262 wherein the sample is a water sample.

270. The method of claim 262 wherein during replication at least one of the replicated strands is displaced from nucleic acids in the sample by strand displacement replication of another replicated strand.

271. The method of claim 262 wherein the nucleic acids are replication using exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), amplification with Qβ replicase, and cycle sequencing.

272. A method of detecting the presence of an organism in a sample, the method comprising,

exposing the sample to alkaline conditions,

reducing the pH of the sample to form a stabilized sample,

incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample, wherein replication of the nucleic acids results in replicated strands, and

identifying one or more types of organism that contain one or more of the sequences of the replicated strands, thereby detecting the presence of the organism in the sample.

273. The method of claim 272 wherein the one or more types of organism that contain one or more of the sequences of the replicated strands are identified by sequencing at least a portion of the replicated strands to obtain one or more sequences of the replicated strands, searching a database of nucleic acid sequences sequentially using one or more of the sequences of the replicated strands as strings, and identifying the results of the search as sequences of organisms likely to be present in the sample and not likely to be present in the sample, thereby detecting the presence of the organism in the sample.

274. The method of claim 273 wherein the sample comprises nucleic acids from a whole ecosystem, wherein at least one organism in the sample is a variant organism, wherein the variant organism comprises a variant sequence from sequence of the same type of organism present in the database, wherein identifying a type of organism that contains one or more of the sequences of the replicated strands identifies at least one organism.

275. The method of claim 273 wherein sequencing at least a portion of the replicated strands is accomplished by hybridization to at least one of a plurality of nucleic acid probes.

276. The method of claim 275 wherein the nucleic acid probes are immobilized on a microarray.

277. The method of claim 272 wherein the sample comprises nucleic acids from two organisms, wherein identifying a type of organism that contains one or more of the sequences of the replicated strands identifies both organisms.

278. The method of claim 272 wherein the sample comprises nucleic acids from two organisms, wherein identifying a type of organism that contains one or more of the sequences of the replicated strands identifies at least one organism.

279. A method of amplifying nucleic acids, the method comprising,

exposing a sample to alkaline conditions,

reducing the pH of all or a portion of the sample to form a stabilized sample, and

incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample,

wherein replication of the nucleic acids results in replicated strands, wherein the replicated strands have low amplification bias, wherein the concentration of nucleic acids in the amplification mixture favors hybridization of primers over reassociation of the nucleic acids, wherein amount of nucleic acids in the amplification mixture is at or above a threshold that can result in low amplification bias in the replicated strands.

280. The method of claim 279 wherein the nucleic acids are replicated using exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), amplification with Qβ replicase, and cycle sequencing.

281. A method of identifying reaction conditions for nucleic acid amplification, the method comprising

amplifying a test sample under test conditions to produce amplified nucleic acids,

measuring amplification bias in the amplified nucleic acids,

wherein if the amplification bias is less than a threshold of interest, then the test conditions are identified as conditions for a nucleic acid amplification.

282. The method of claim 281wherein the nucleic acids are replicated using exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification

(TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), amplification with Qβ replicase, and cycle sequencing.

283.  A method of identifying reaction conditions for nucleic acid amplification, the method comprising

exposing a test sample to alkaline conditions,

reducing the pH of all or a portion of the test sample to form a stabilized test sample, and

incubating a test amplification mixture under conditions to produce amplified nucleic acids, wherein the test amplification mixture comprises all or a portion of the test stabilized sample,

wherein the test conditions promote replication of nucleic acids, wherein the concentration of nucleic acids in the test amplification mixture is a test nucleic acid concentration, wherein the amount of nucleic acids in the test amplification mixture is a test amount of nucleic acids,

measuring amplification bias in the amplified nucleic acids,

wherein if the amplification bias is less than a threshold of interest, then the test concentration of nucleic acids is a concentration to be used for nucleic acid amplification and the test amount of nucleic acids is threshold amount to be used for nucleic acid amplification.

284.  A method of amplifying nucleic acids, the method comprising,

exposing a sample that may comprise nucleic acids to alkaline conditions,

reducing the pH of all or a portion of the sample to form a stabilized sample, and

incubating an amplification mixture under conditions that promote replication of the nucleic acids from the sample, wherein the amplification mixture comprises all or a portion of the stabilized sample,

wherein replication of the nucleic acids results in replicated strands, wherein during replication at least one of the replicated strands is displaced from nucleic acids in the sample by strand displacement replication of another replicated strand, wherein the replicated strands have an amplification bias less than a threshold of interest,

wherein the concentration of nucleic acids in the amplification mixture is at or above a predetermined concentration, wherein amount of nucleic acids in the amplification mixture is at or above a threshold amount, wherein the predetermined concentration and the threshold amount are determined by

exposing a test sample to the alkaline conditions,

347

reducing the pH of all or a portion of the test sample to form a stabilized test sample, and

incubating a test amplification mixture under conditions that promote replication of nucleic acids to produce amplified nucleic acids, wherein the amplification mixture comprises all or a portion of the stabilized sample,

wherein the concentration of nucleic acids in the test amplification mixture is a test nucleic acid concentration, wherein the amount of nucleic acids in the test amplification mixture is a test amount of nucleic acids,

measuring amplification bias in the amplified nucleic acids,

wherein if the amplification bias is less than the threshold of interest, then the test concentration of nucleic acids is the predetermined concentration and the test amount of nucleic acids is the threshold amount.

285. A kit for amplifying a whole genome, the kit comprising

a stabilization solution,

a reaction mix comprising a set of primers, and

a DNA polymerase mix comprising a DNA polymerase.

286. The kit of claim 285 further comprising

1M dithiotheitol,

1X Phosphase-Buffered Saline, pH 7.5, and

control DNA template,

wherein the stabilization solution comprises 800 mM Tris-HCl, pH 4,

wherein the reaction mix comprises 150 mM Tris-HCl, 200 mM KCl, 40 mM MgCl$_2$, 20 mM (NH$_4$)$_2$SO$_4$, 4 mM deoxynucleotide triphosphates, and 0.2 mM random hexamer primers,

wherein the DNA polymerase is $\phi$29 DNA polymerase.

SUMMARY OF 47 LOCI REPRESENTATION WITH A SINGLE PRIMER (AluR 11)

PRIMER USED (1)

AGCGAG

FIG.1

2/27
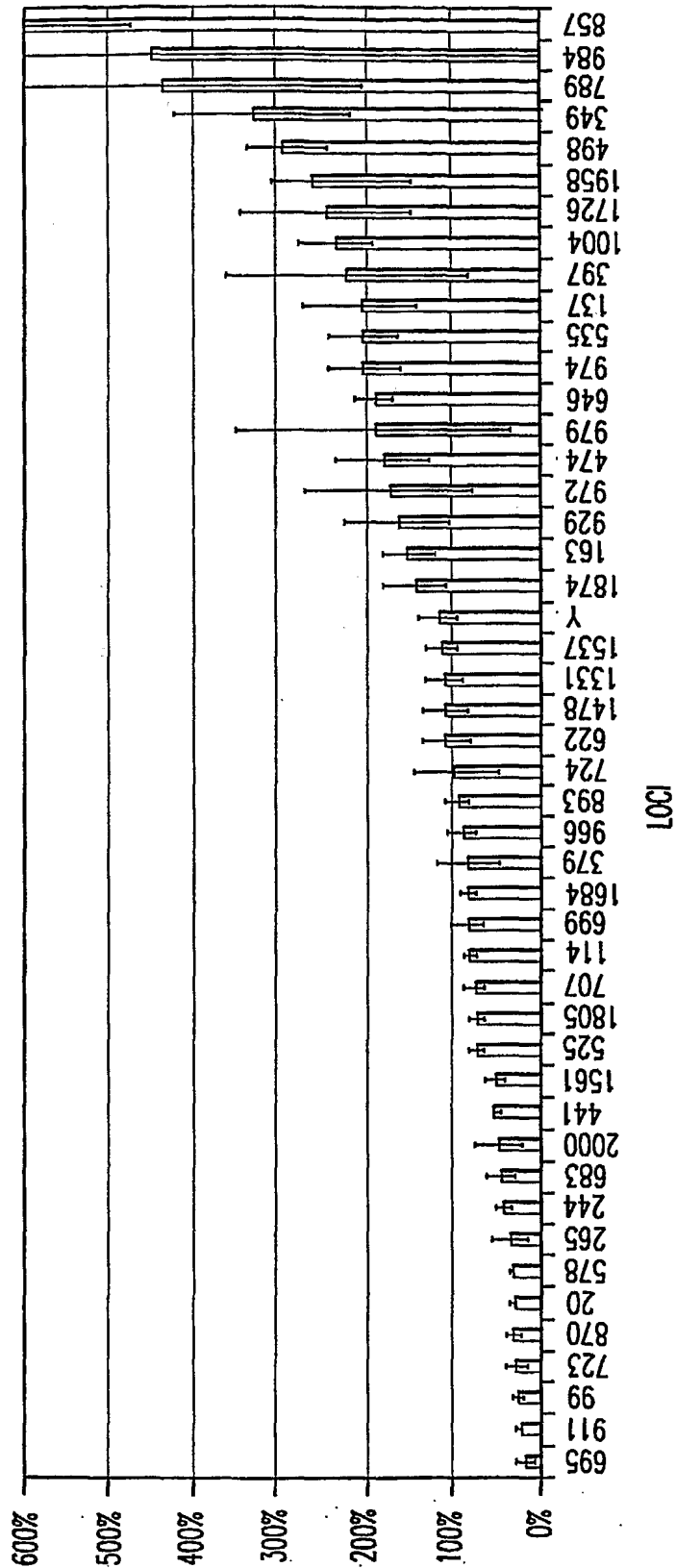


SUMMARY OF 47 LOCI REPRESENTATION WITH A PREFERRED PRIMER SET WITH 2 PRIMERS

PRIMERS USED (2)

AGTGGG
AGAGAG

FIG.2

FIG.3

SUMMARY OF 47 LOCI REPRESENTATION WITH A PREFERRED PRIMER SET WITH 3 PRIMERS
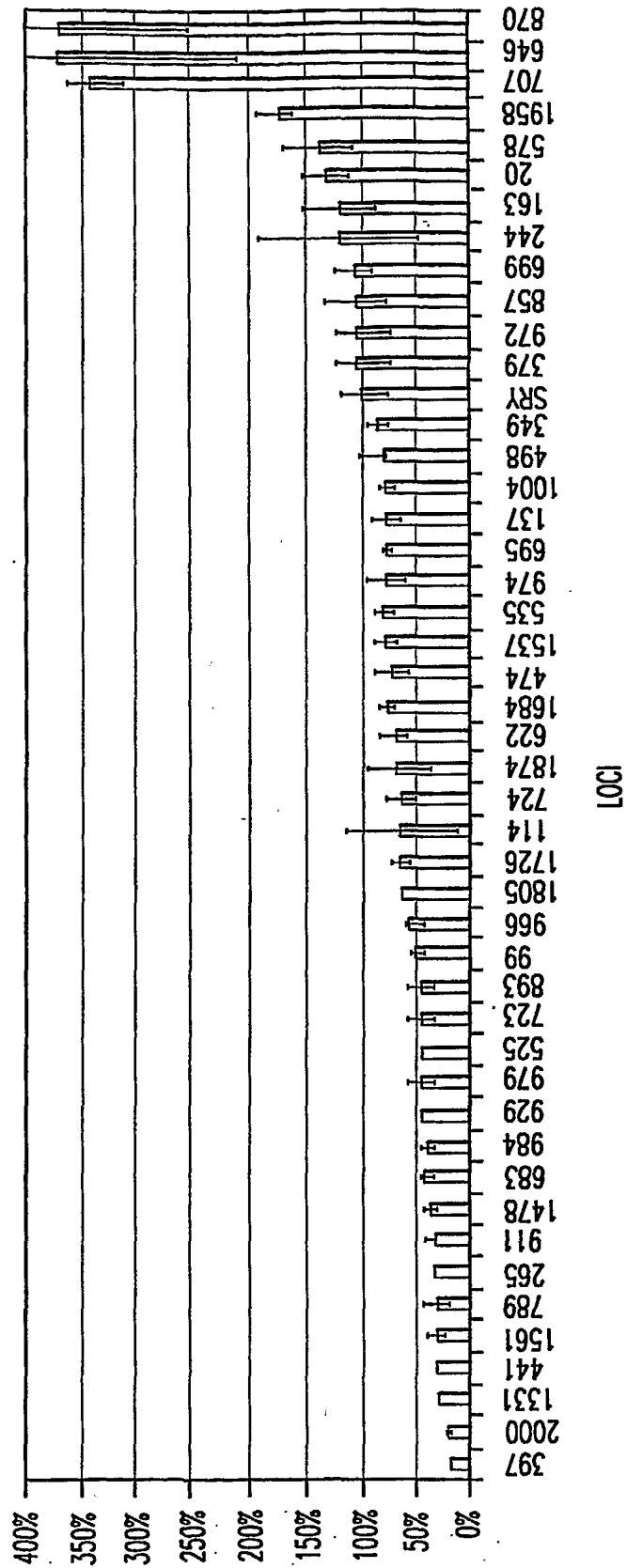
PRIMERS USED (3)

AGCCGG
AGTAGG
AGTTGG

LOCI

FIG.4

FIG.5

SUMMARY OF 47 LOCI REPRESENTATION WITH A PREFERRED PRIMER SET WITH 5 PRIMERS

PRIMERS USED (5)

AGAGGG
AGGCAG
AGCCAG
AGTCAG
AGACAG
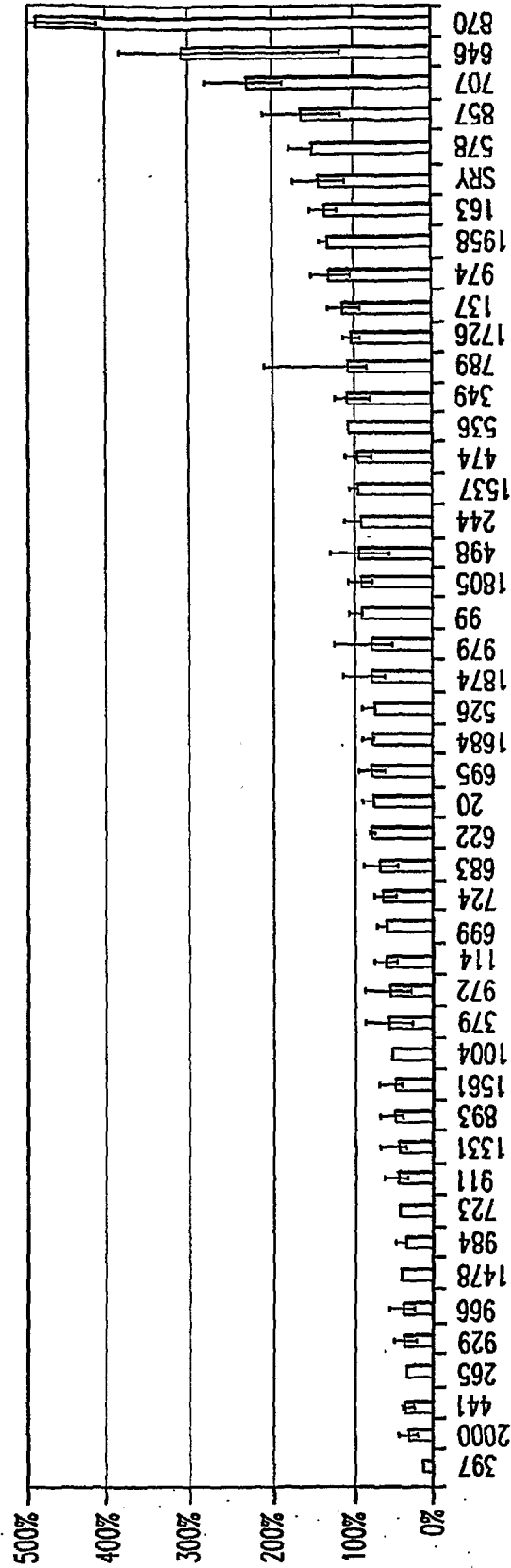
FIG.6

PRIMERS USED (5)

AGTAGG
AGGTGG
AGGCAG
AGACAG
AGTGAG

SUMMARY OF 47 LOCI REPRESENTATION WITH A PREFERRED PRIMER SET WITH 5 PRIMERS
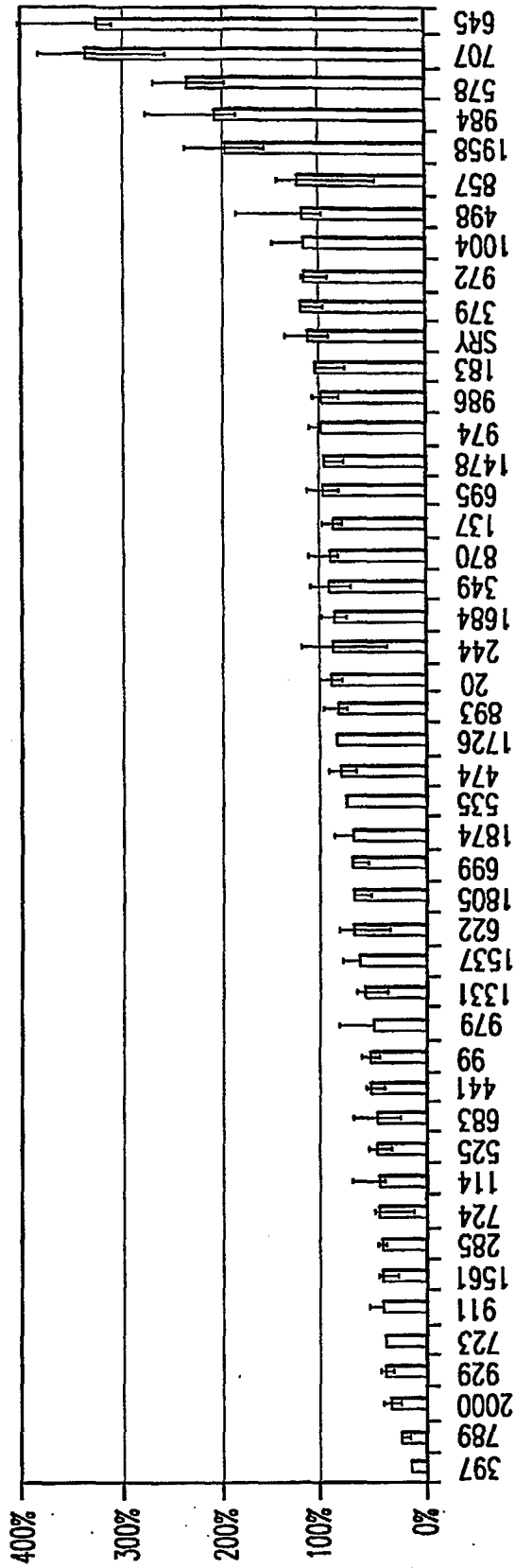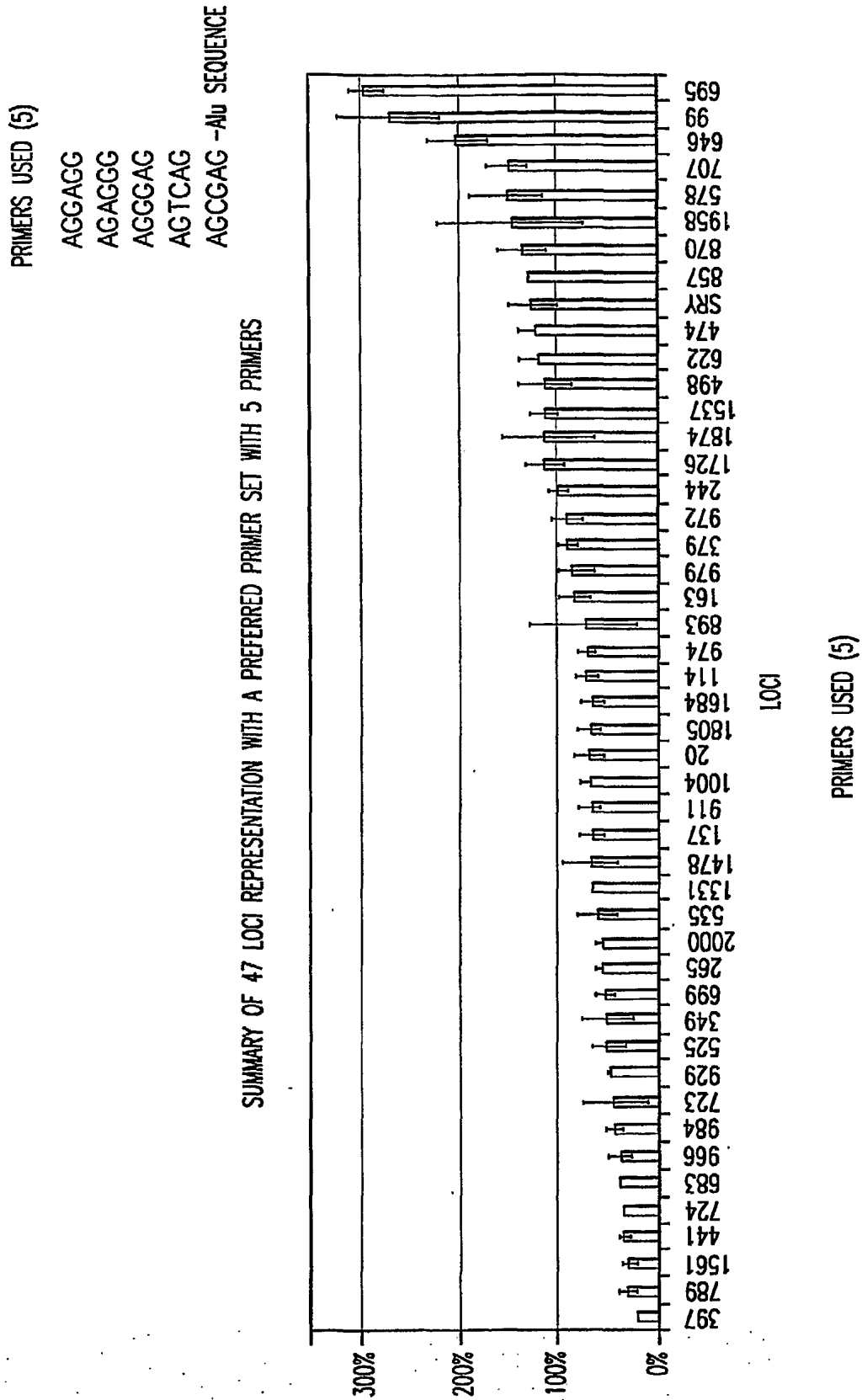
LOCI

FIG.7

SUMMARY OF 47 LOCI REPRESENTATION WITH A PREFERRED PRIMER SET WITH 5 PRIMERS

PRIMERS USED (5)

AGGAGG
AGAGGG
AGGGAG
AGTCAG
AGCGAG -Alu SEQUENCE

FIG.8
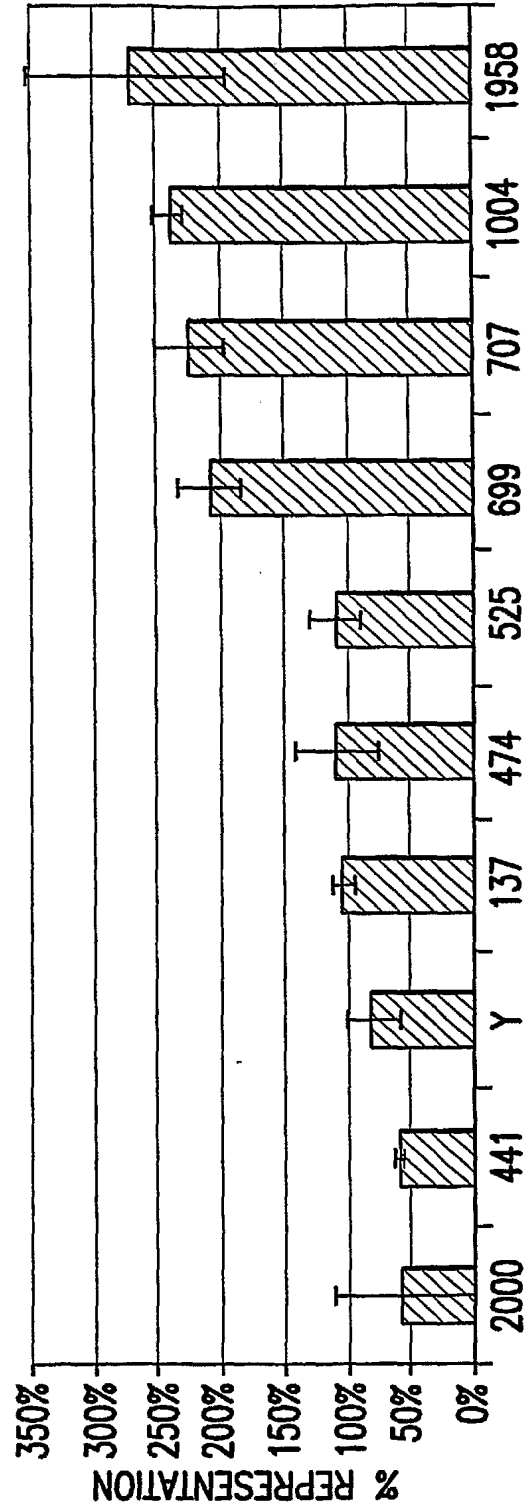
FIG.9
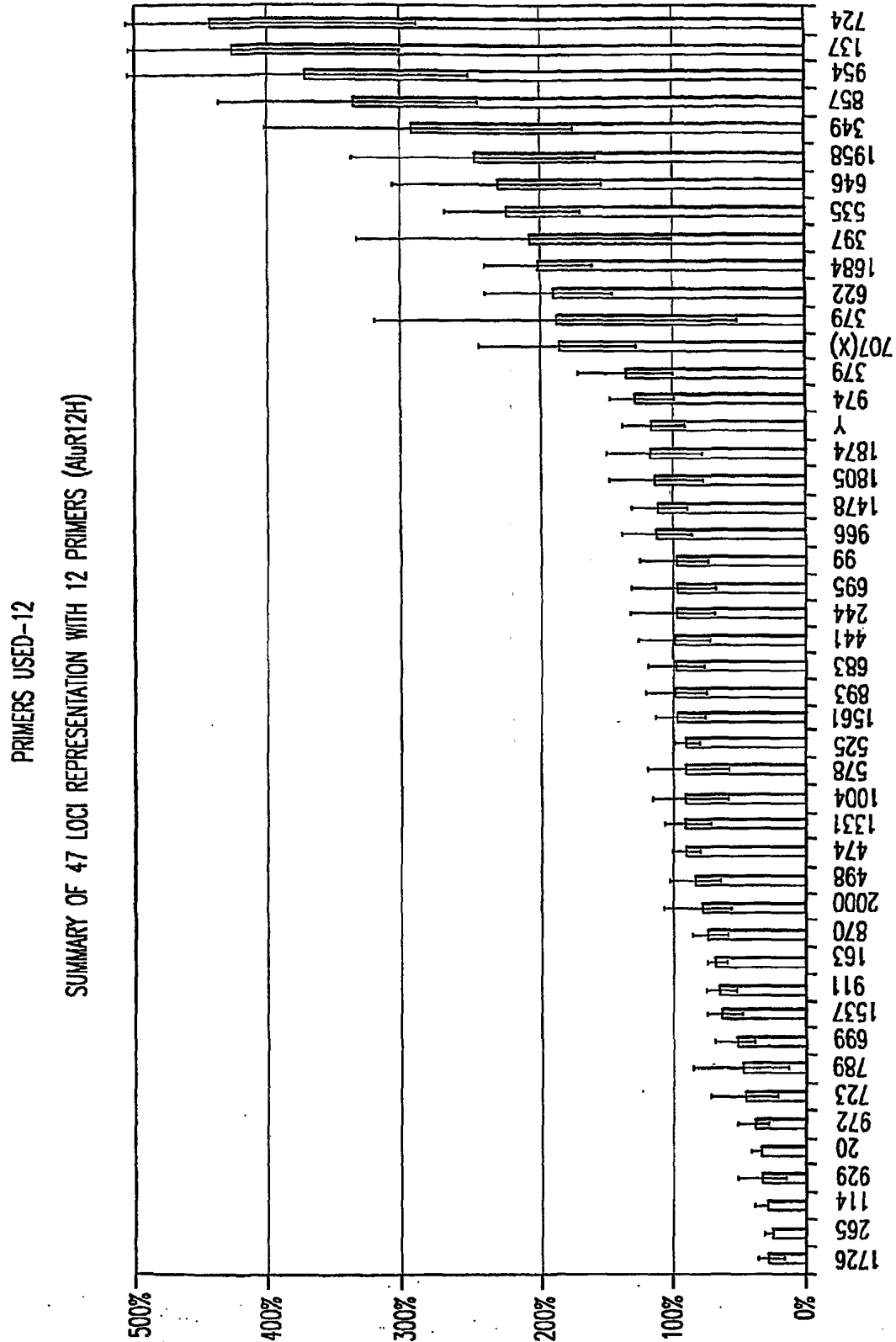
FIG. 10

16 hrs MDA @ 30° C
NO TREHALOSE
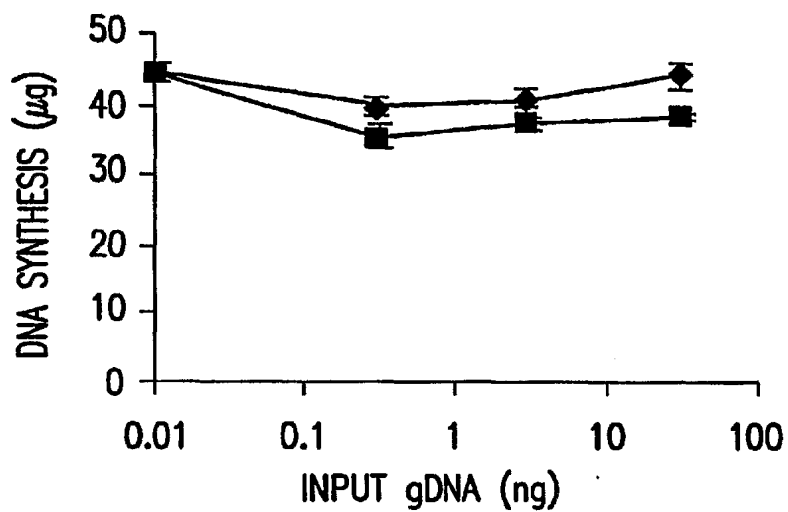


FIG.11A

16 hrs MDA @ 40° C
0.3M TREHALOSE
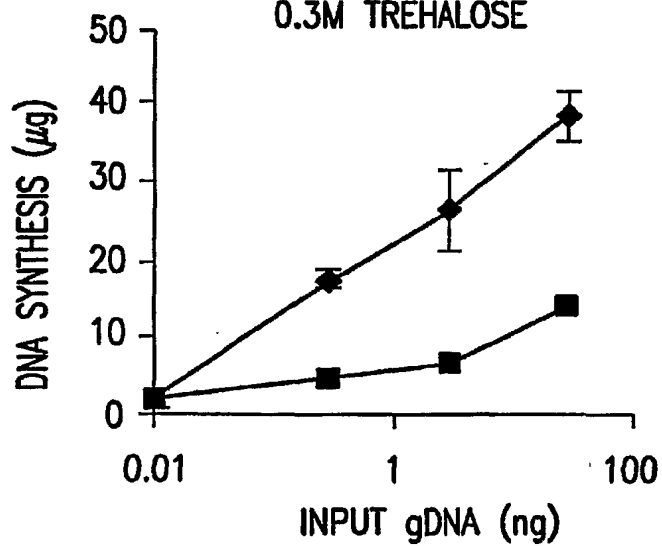


FIG.11B

12/27
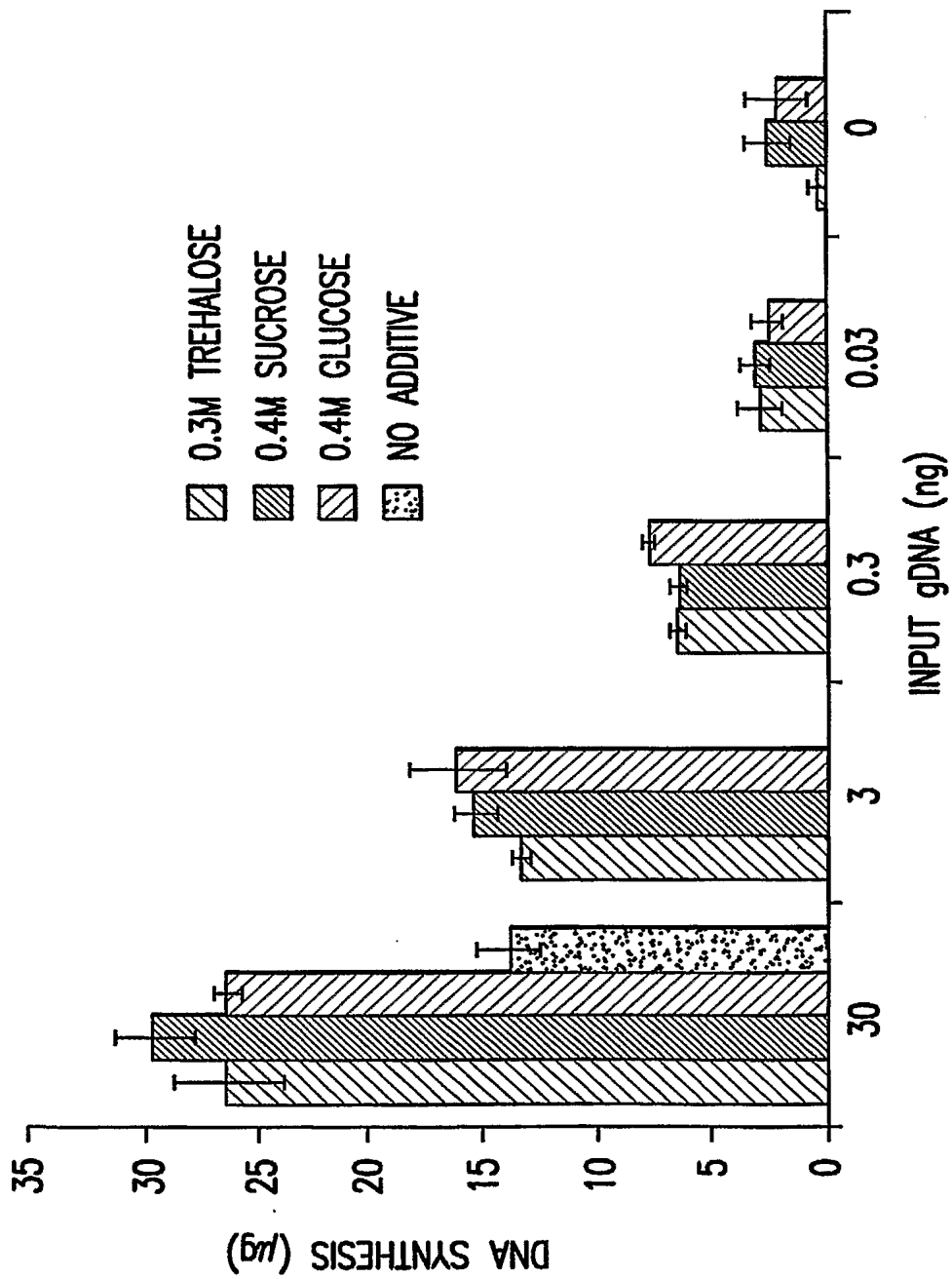


FIG.12
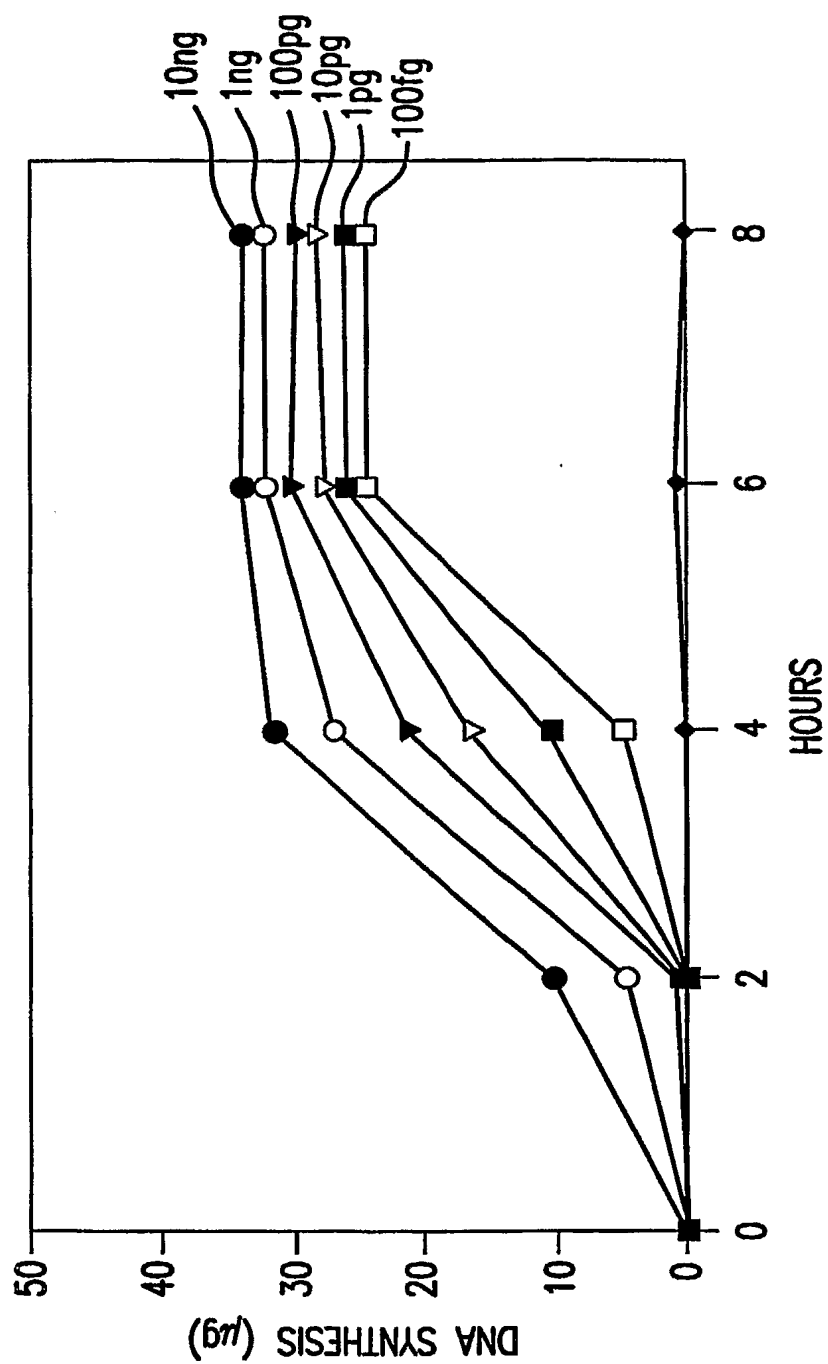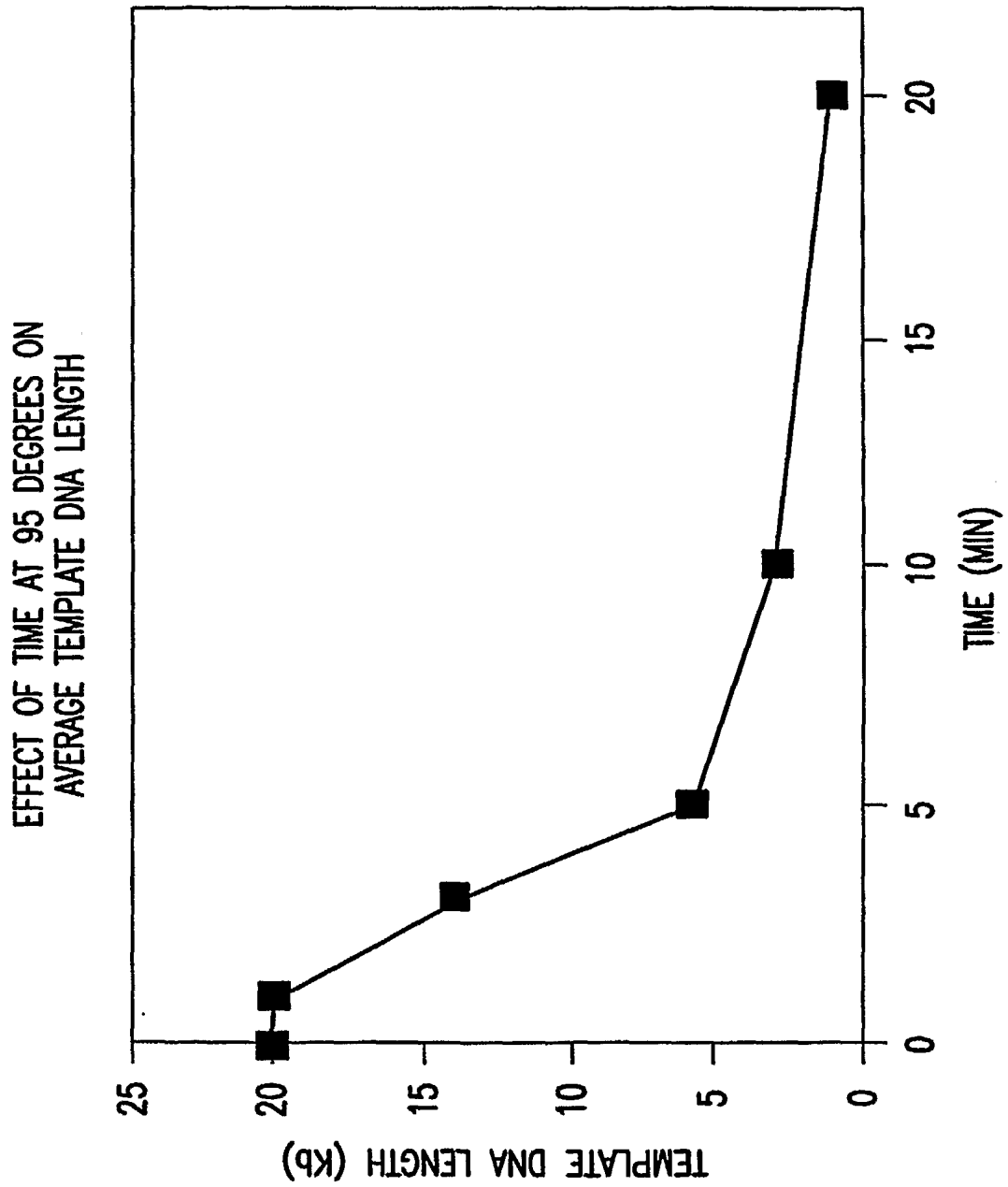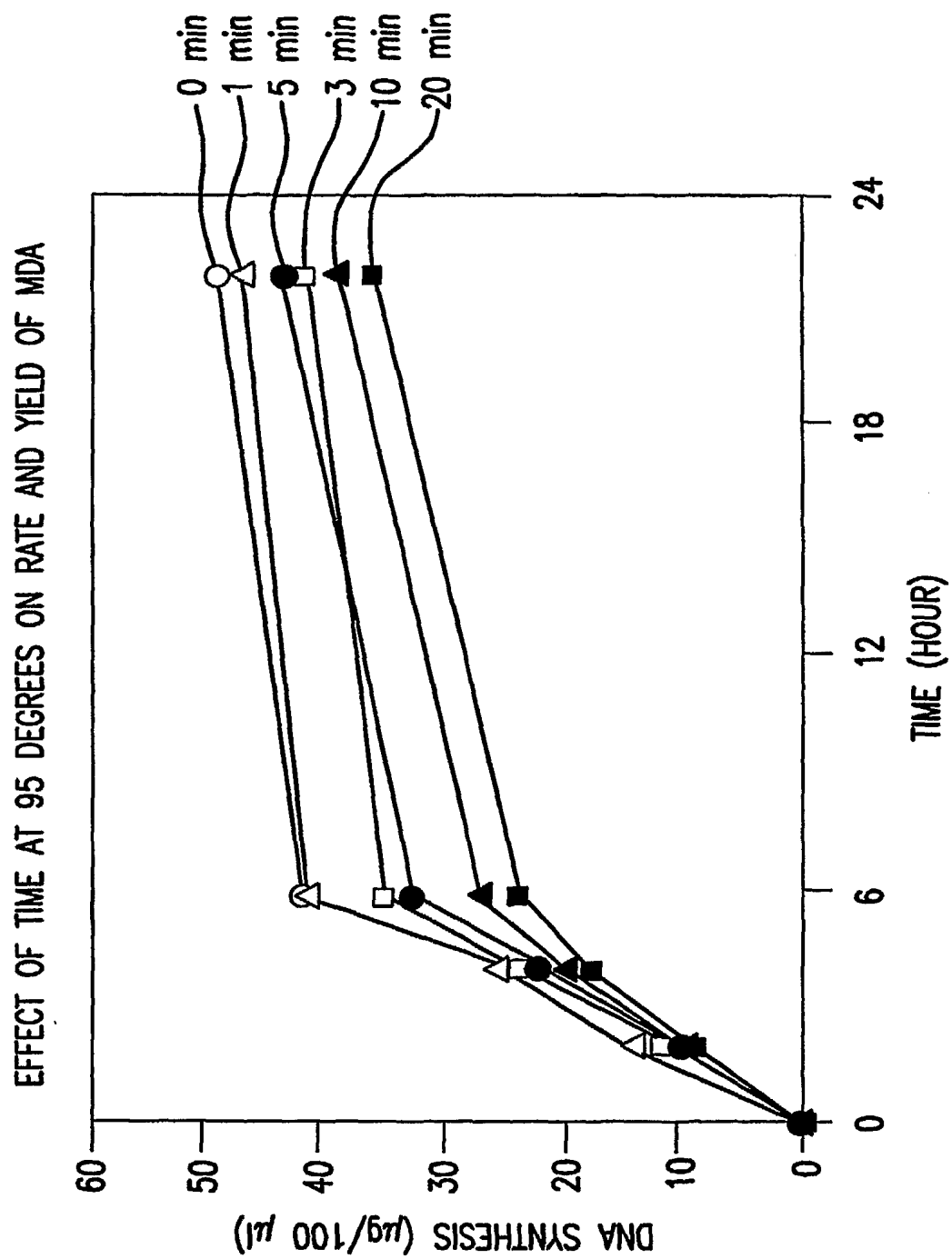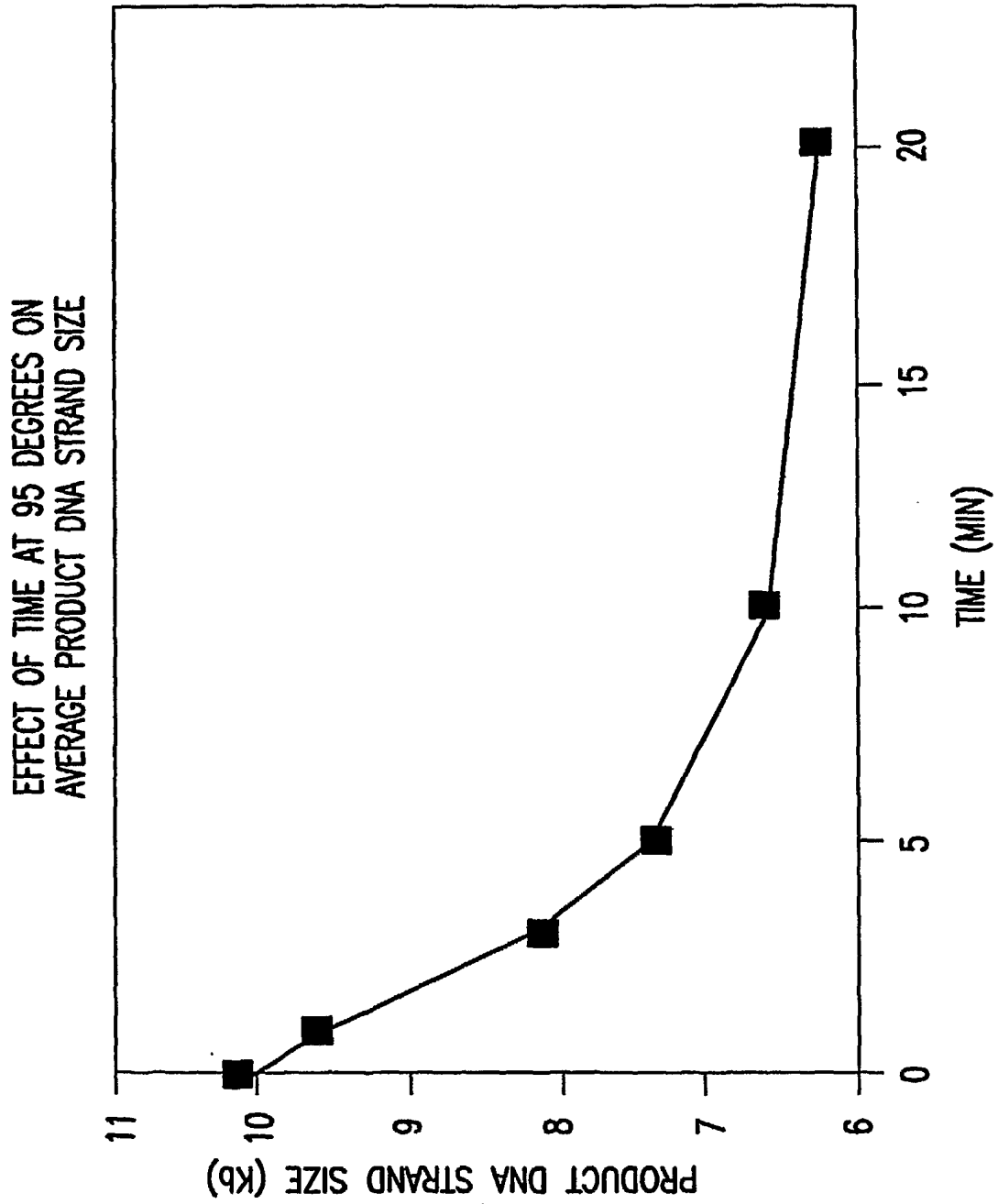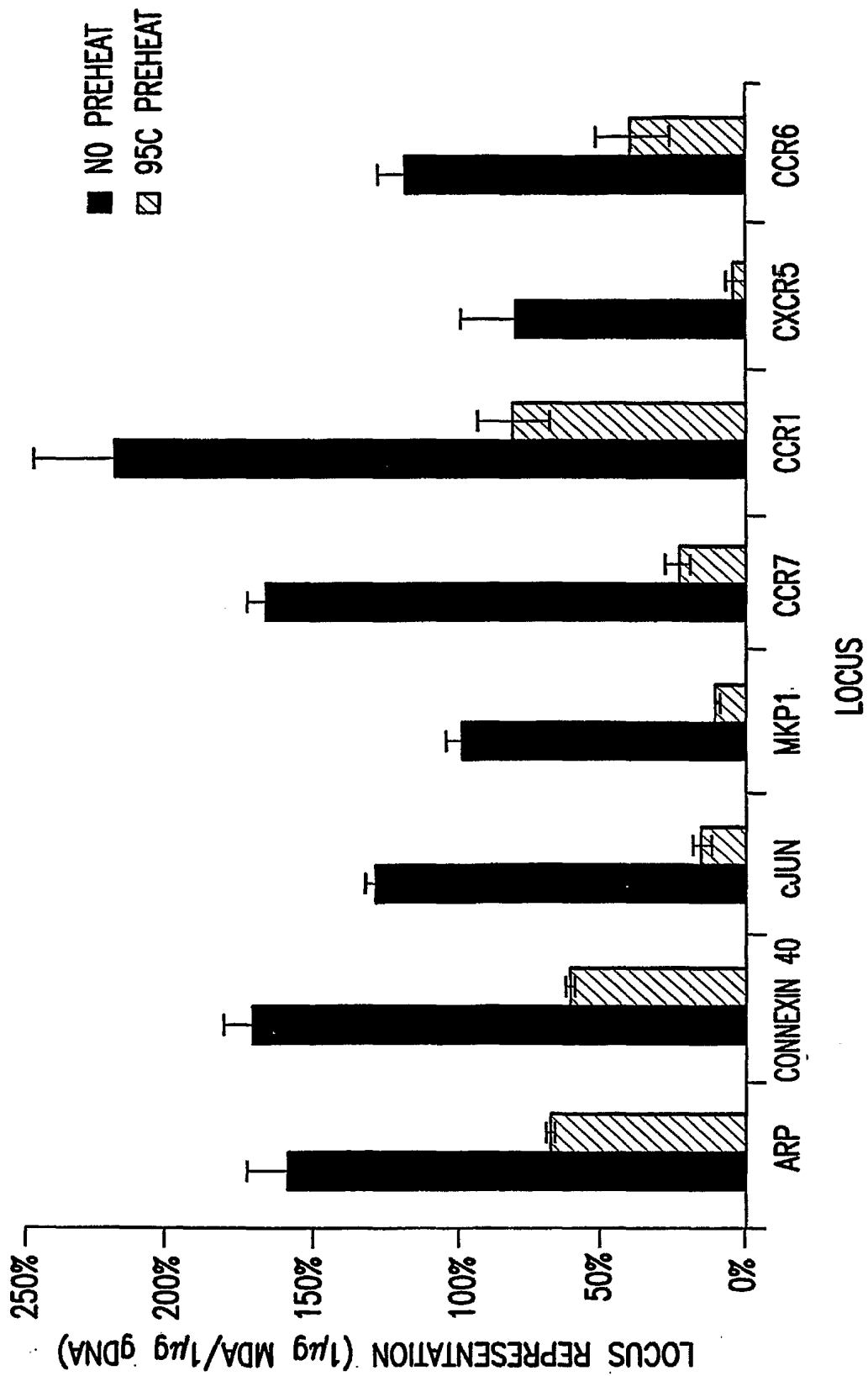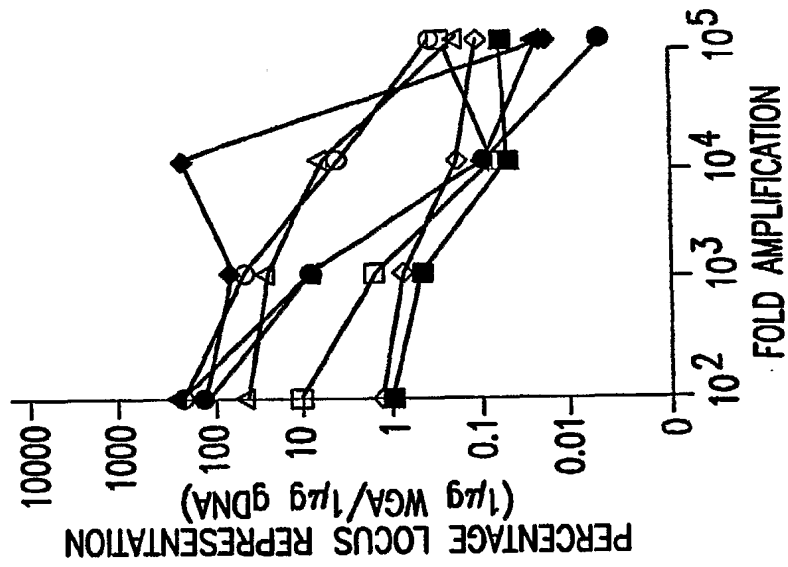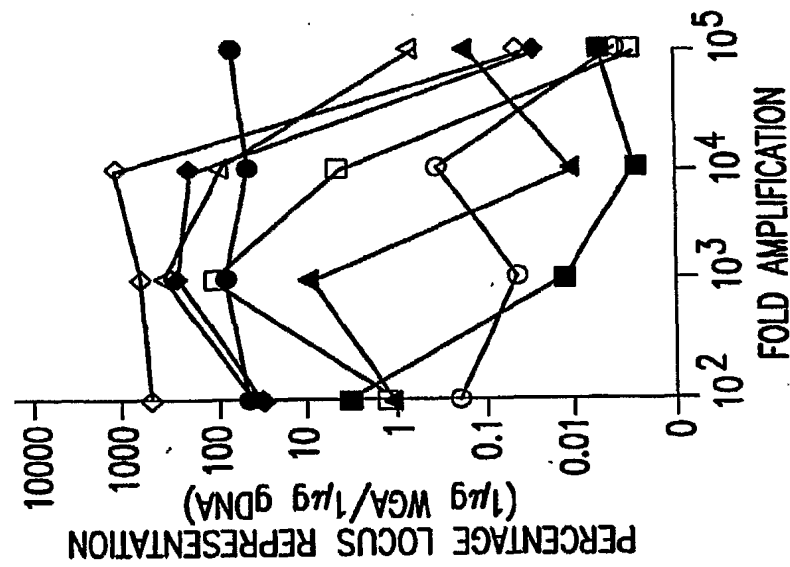
FIG.13

14/27

EFFECT OF TIME AT 95 DEGREES ON
AVERAGE TEMPLATE DNA LENGTH



FIG.14

FIG.15

EFFECT OF TIME AT 95 DEGREES ON
AVERAGE PRODUCT DNA STRAND SIZE

FIG.16

FIG.17
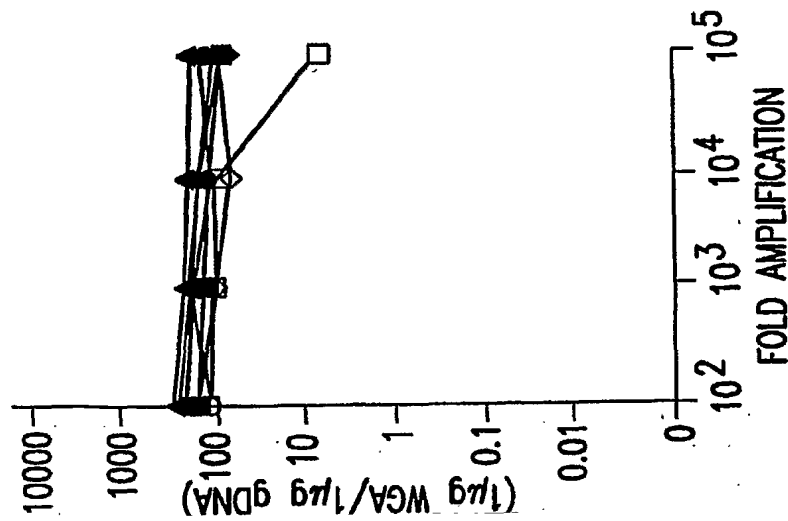
FIG.18C

FIG.18B

FIG.18A

19/27
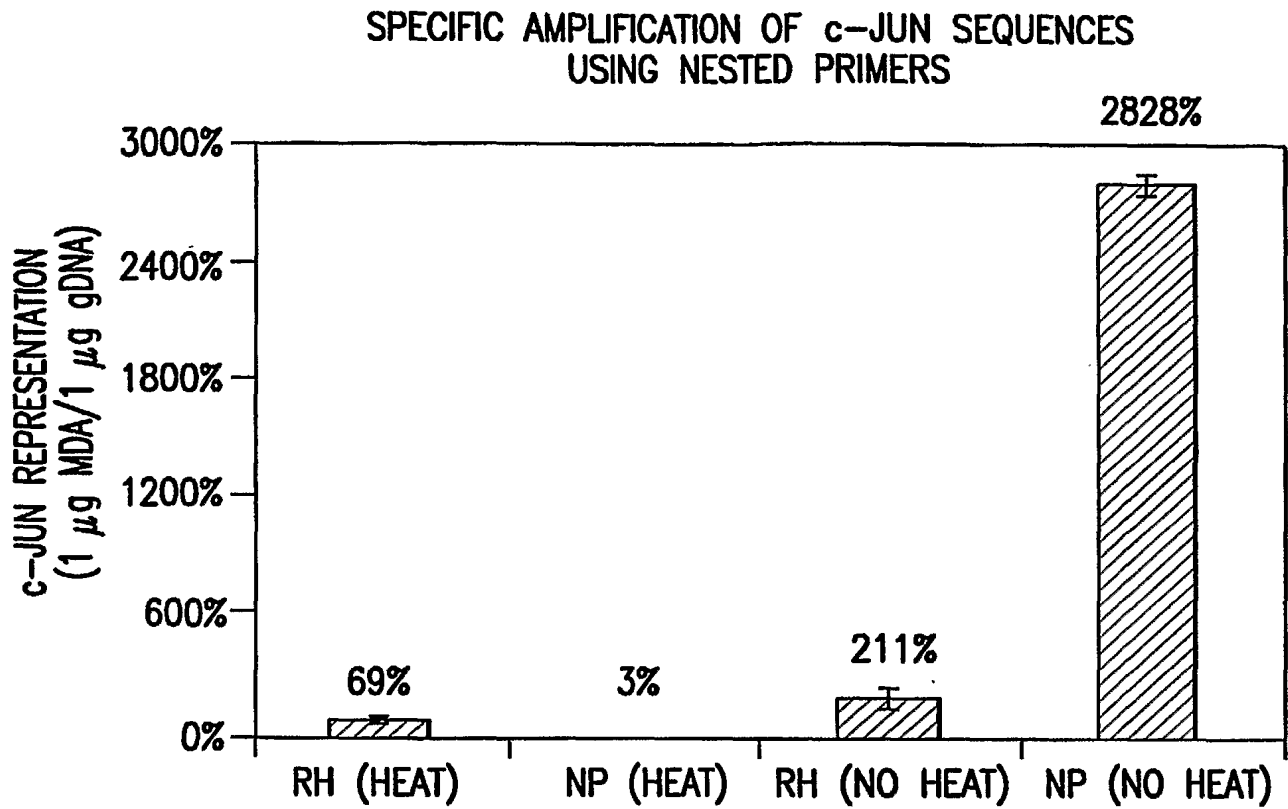


FIG.19
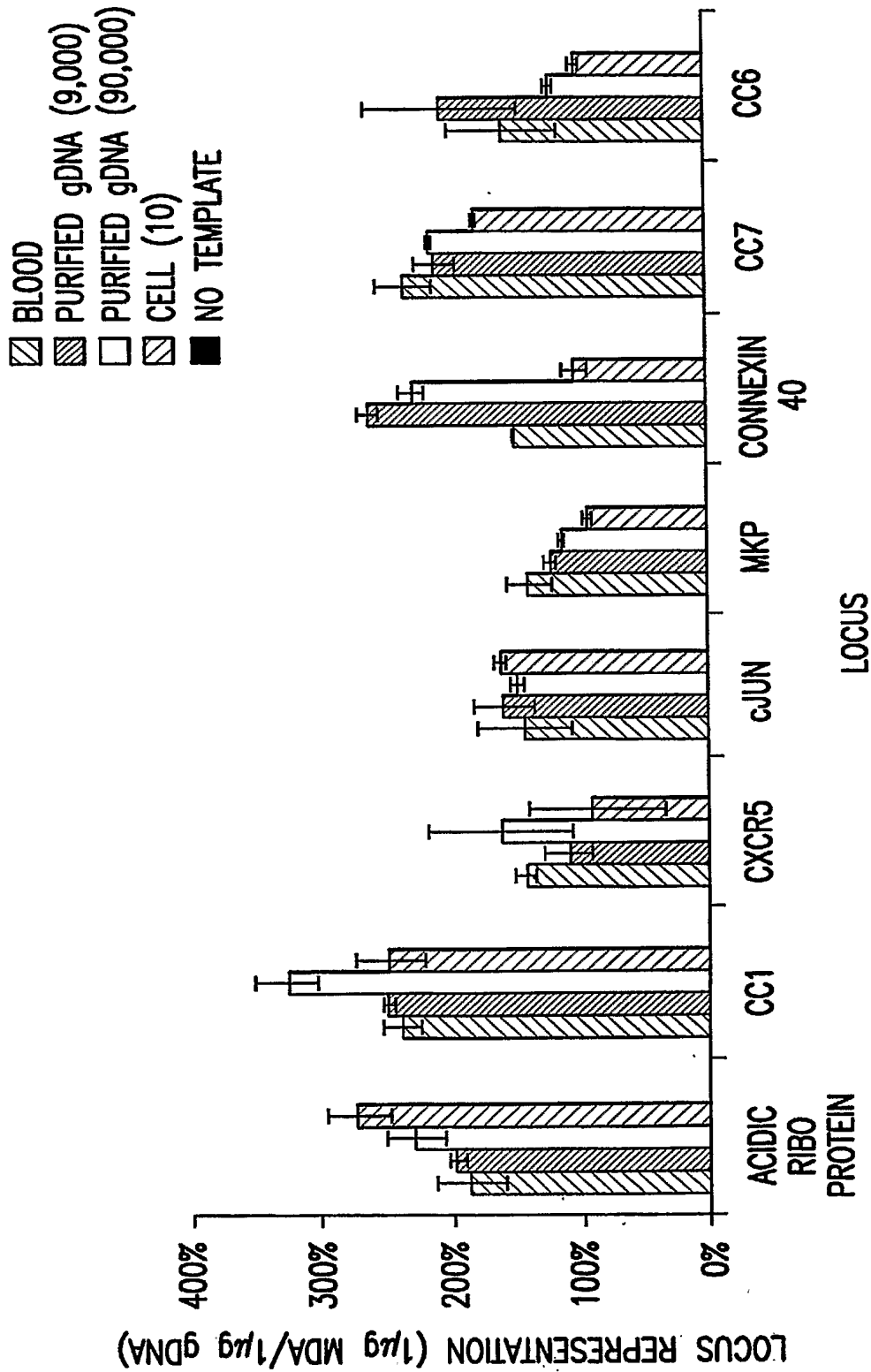
FIG.20
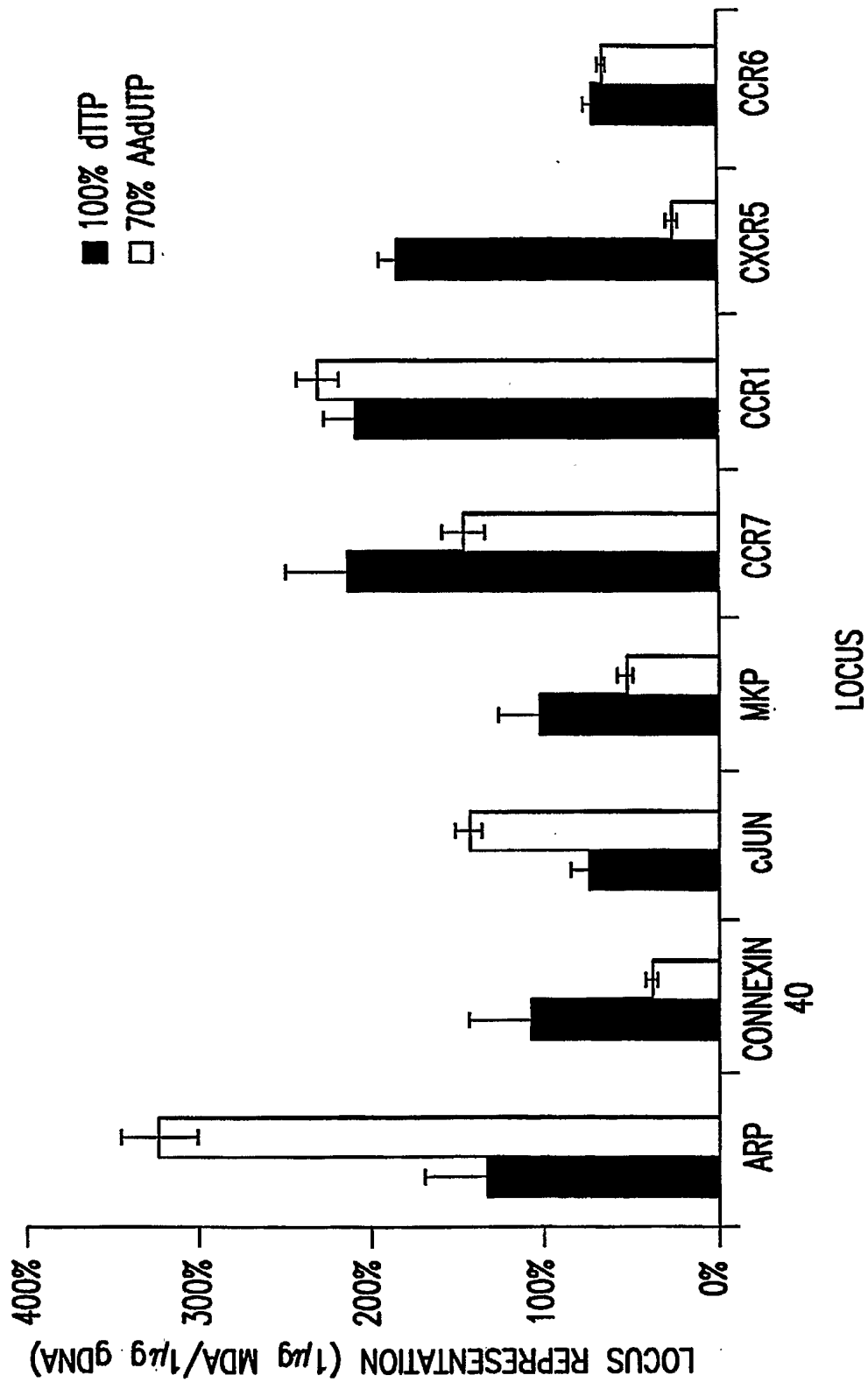
FIG.21
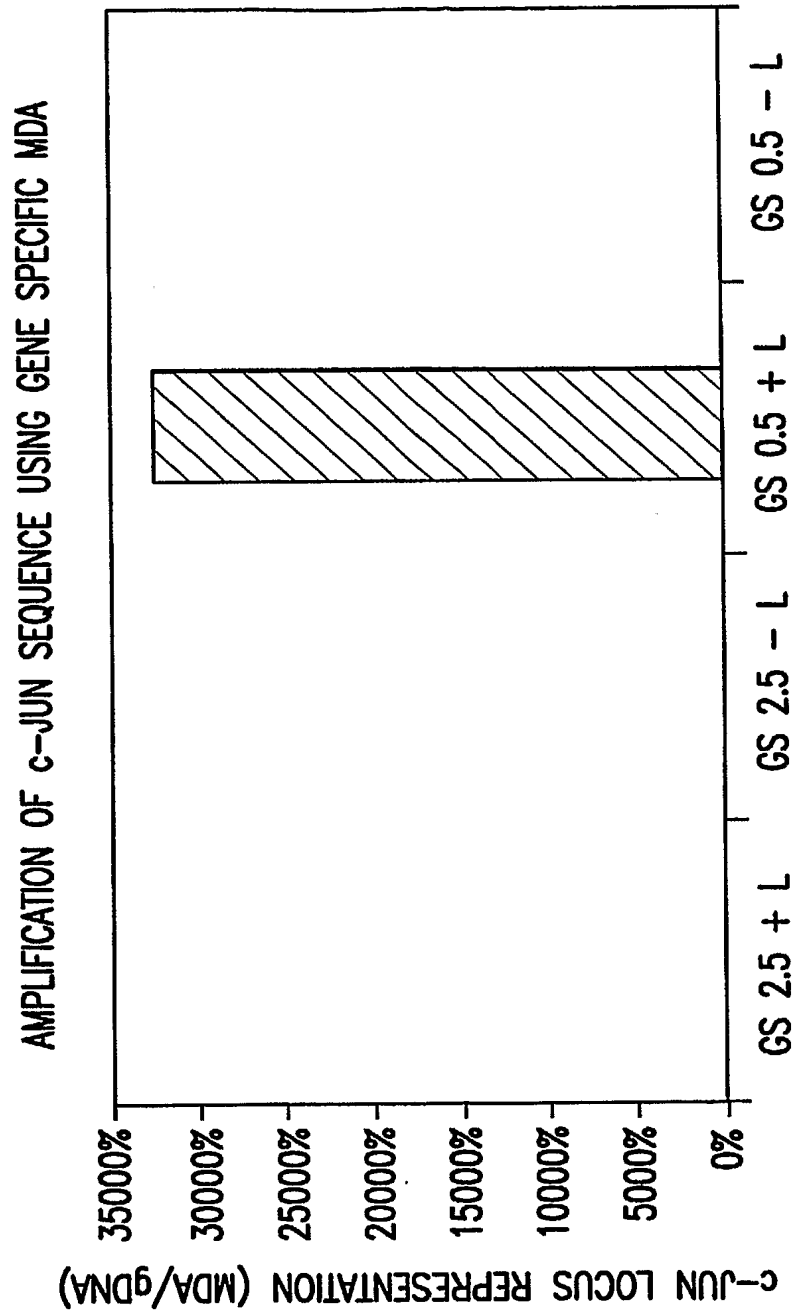
FIG.22

23/27
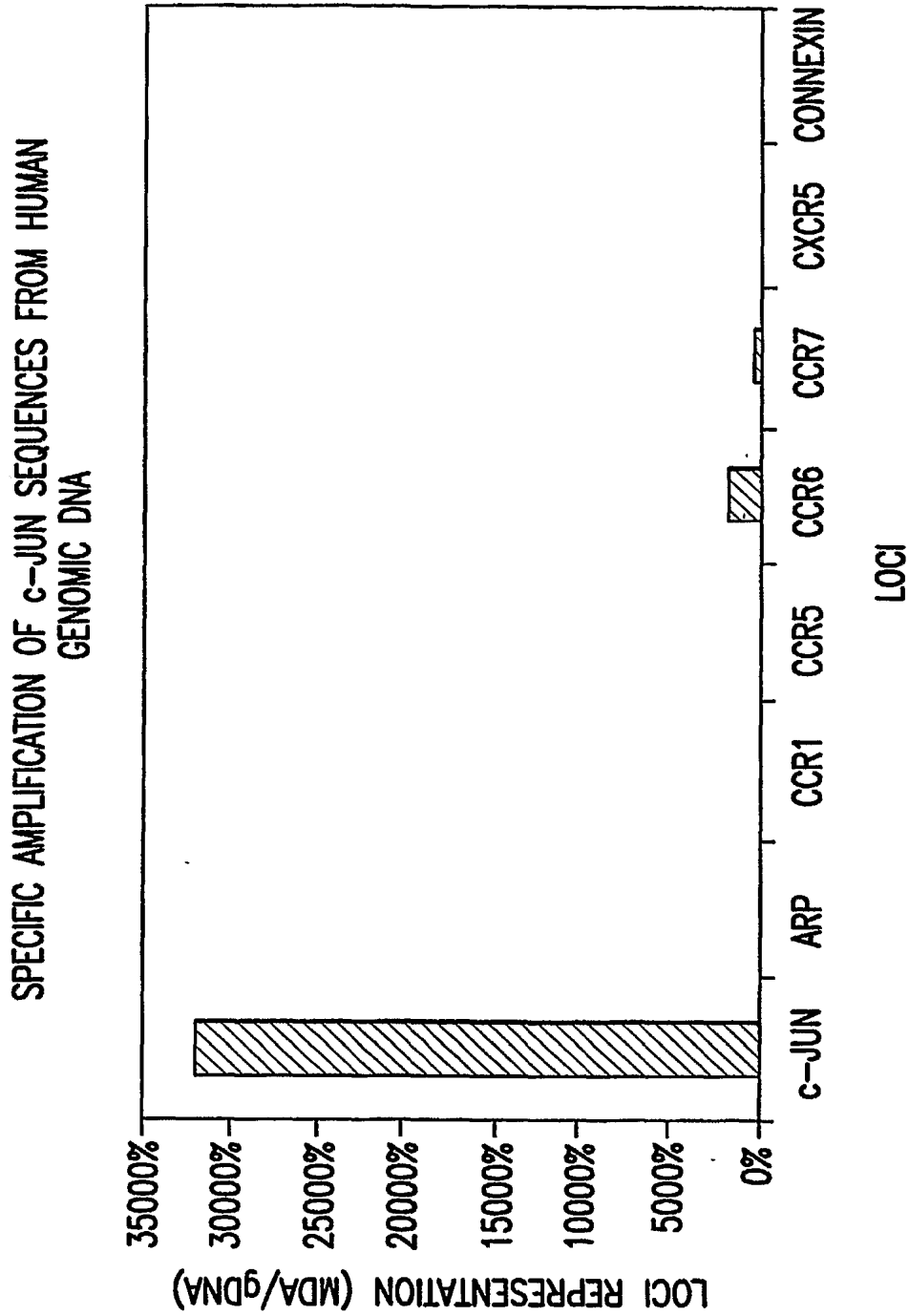


FIG.23
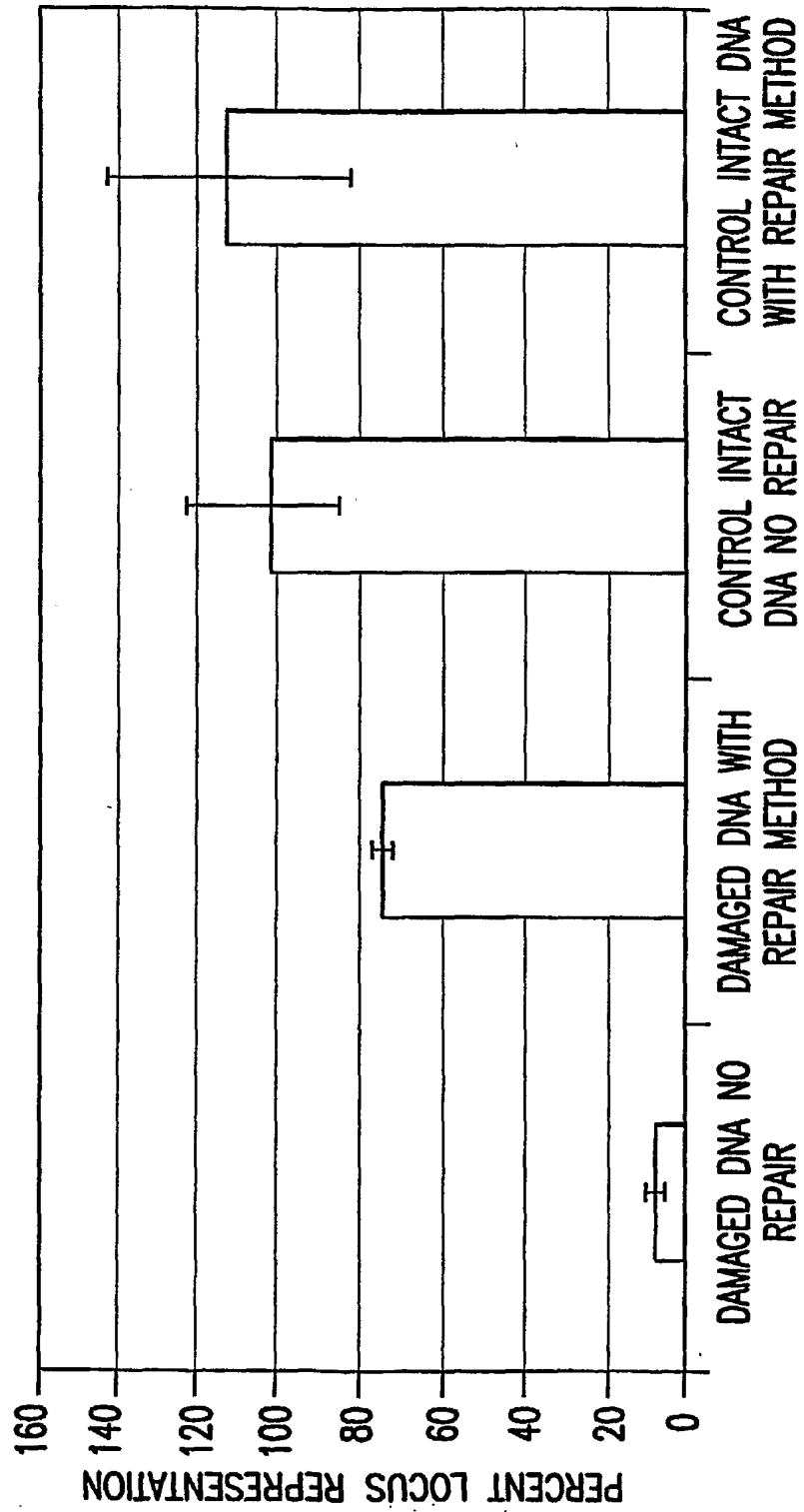
FIG.24

TAQMAN ANALYSIS OF MULTIPLE DISPLACEMENT AMPLIFICATION OF 40 SAMPLES WITH OR WITHOUT THE RECOVERY TREATMENT
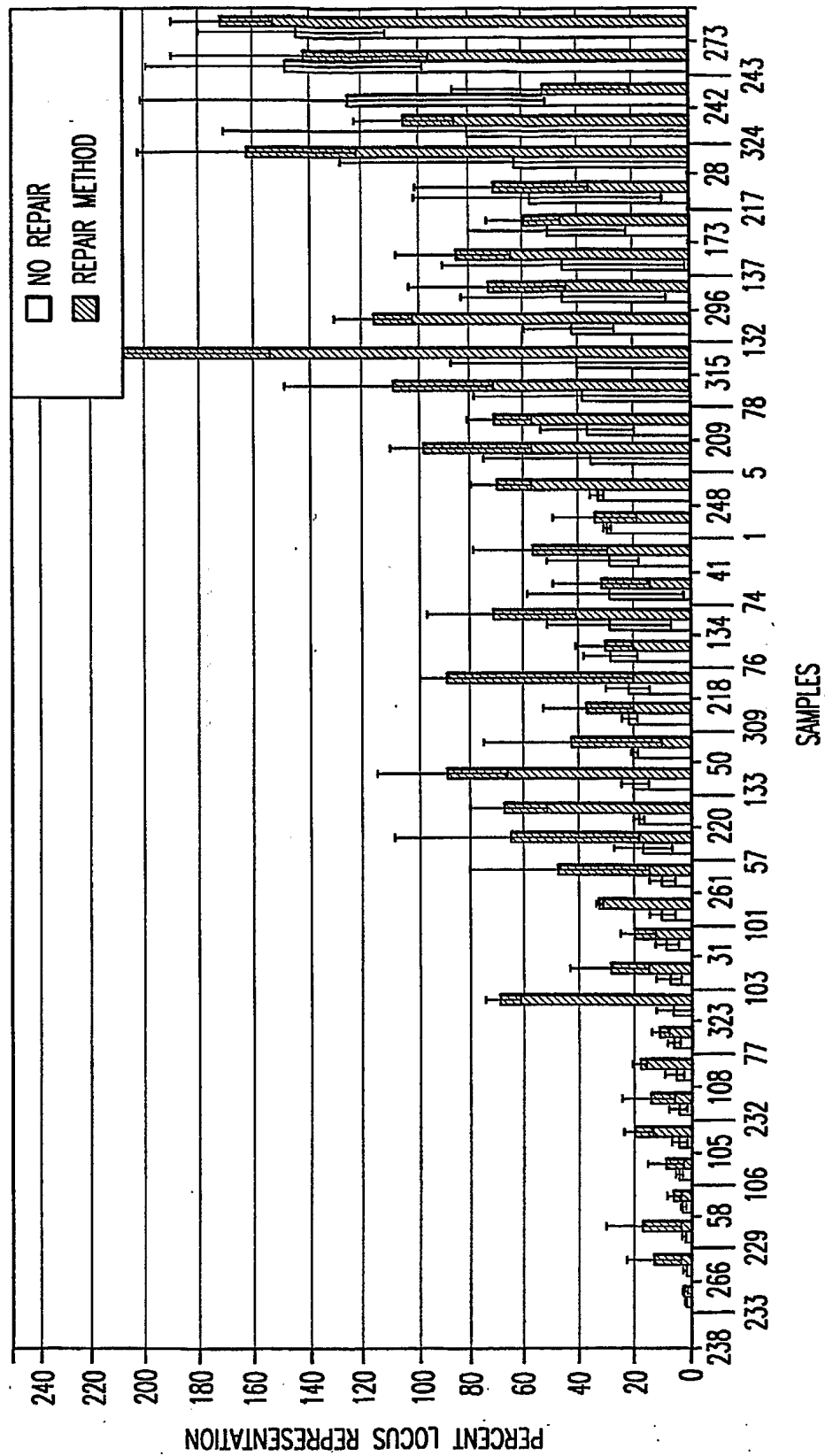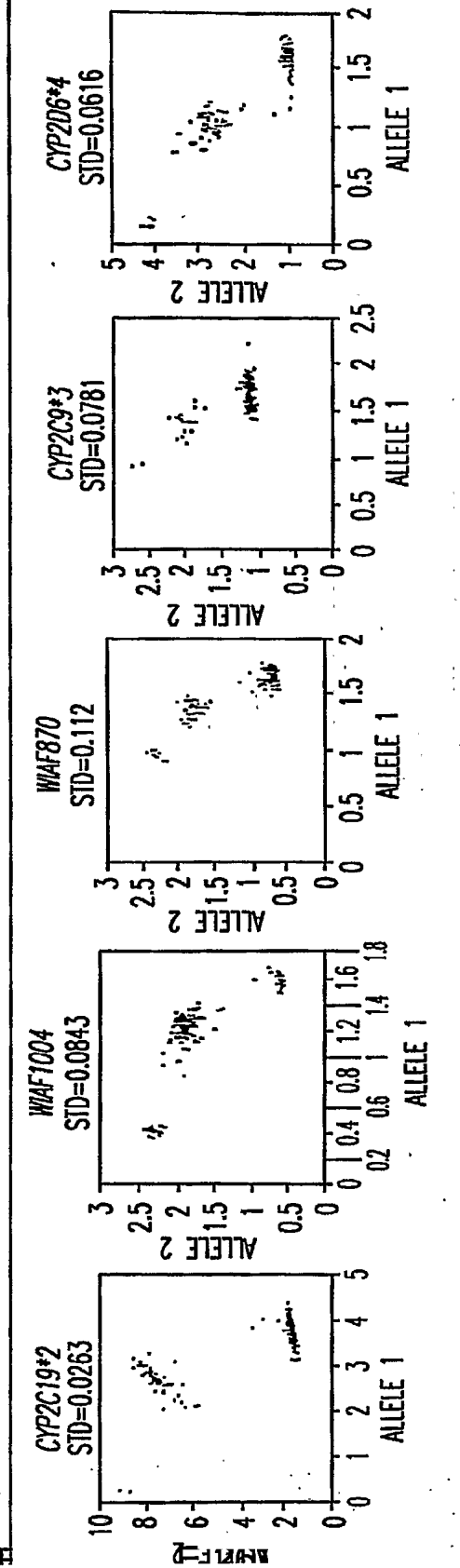
FIG.25
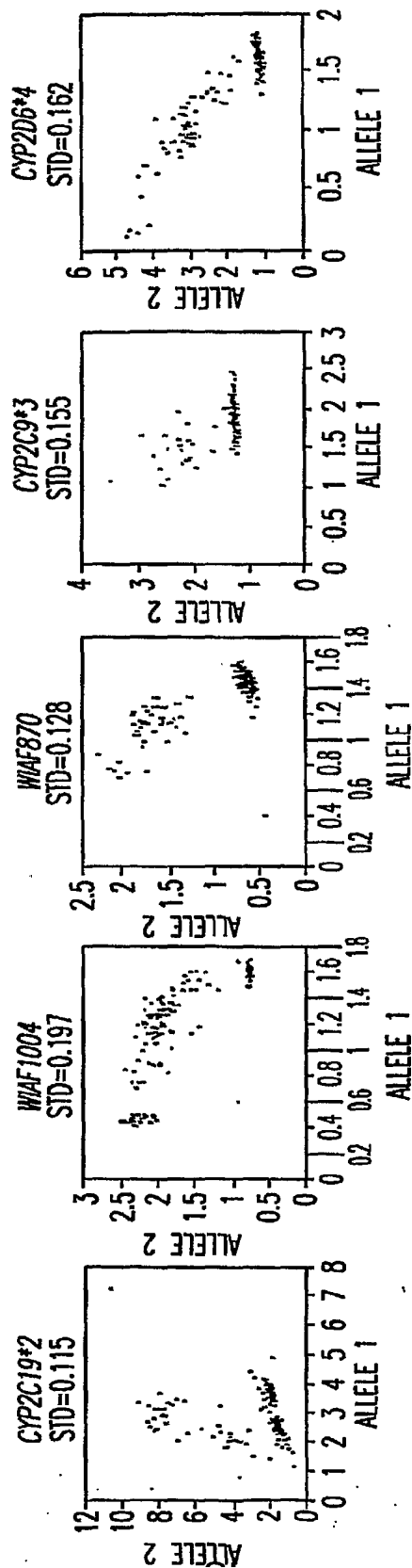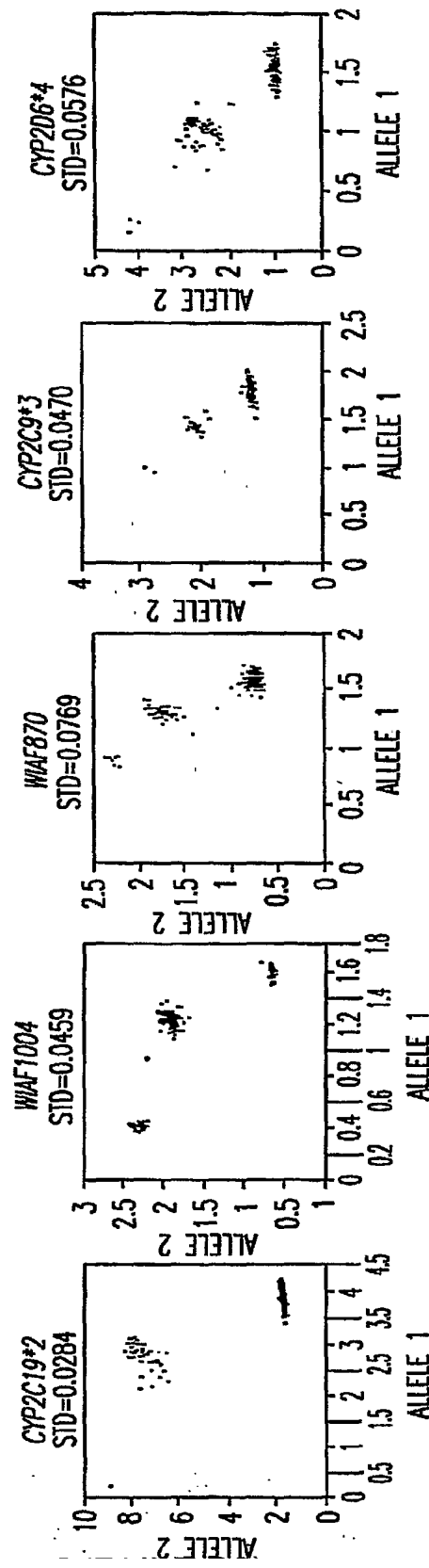
FIG.26
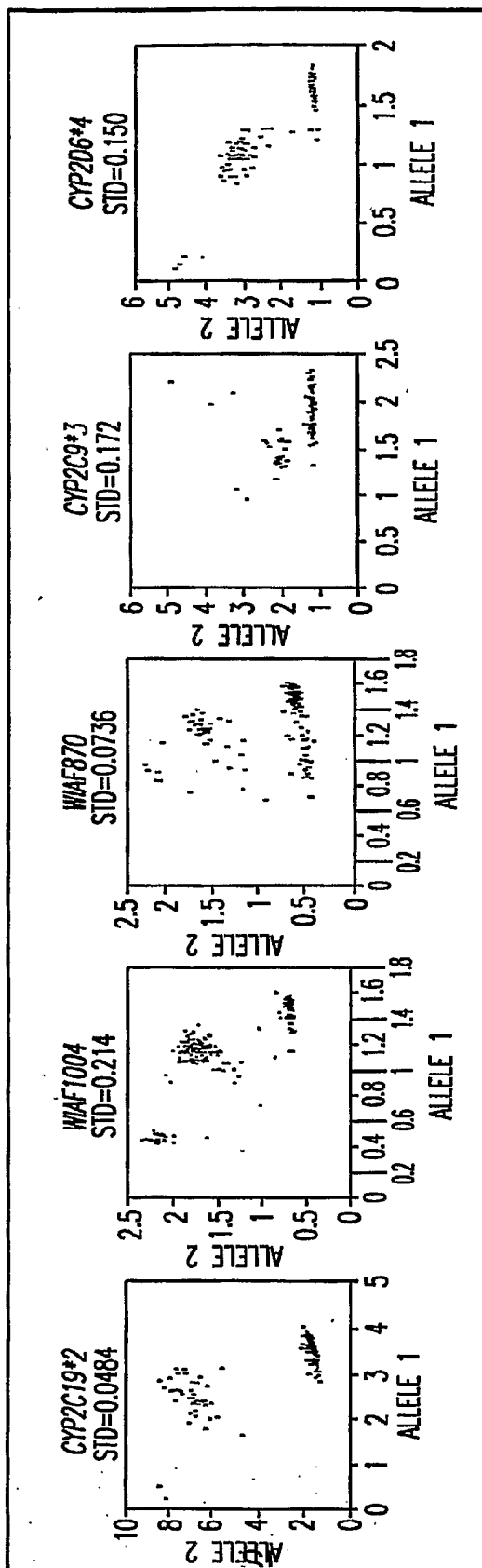
FIG.27

SEQUENCE LISTING

<110> Molecular Staging Inc.

    Bornarth, Carole
    Wisniewski, Michele
    Hosono, Seiyu
    Raghunathan, Arumugham
    Lasken, Roger S.
    Egholm, Michael
    Alsmadi, Osama A.
    Kumar, Gyanendra

<120> NUCLEIC ACID AMPLIFICATION


<130> 13172.0022P1

<140> Unassigned
<141> 2003-12-19

<150> 10/456,056
<151> 2003-06-06

<150> 10/429,229
<151> 2003-05-02

<150> 10/327,602
<151> 2002-12-20

<160> 40

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
    synthetic construct
<400> 1
tccatcacga gttatgcac                                                        19

<210> 2
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
    synthetic construct
<400> 2
tggagttact aagggaagc                                                        19

<210> 3
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =

synthetic construct

<400> 3
actgagttca tgaaccctc                                                              19

<210> 4
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 4
attaactcat tgaaggccc                                                              19

<210> 5
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 5
tctgtgctgt actgttgtc                                                              19

<210> 6
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 6
agtttggcaa actgggctc                                                              19

<210> 7
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 7
tggctcttgg tatgaaaag                                                              19

<210> 8
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 8
actgttagtt tccataggc                                                              19

<210> 9
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =

synthetic construct

<400> 9
tgaatacatt tattgtgac                                                    19


<210> 10
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 10
cgactgtagg agggcagcg                                                    19


<210> 11
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 11
cgtcagccca caatgcacc                                                    19


<210> 12
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 12
gtacttggat tctcagcct                                                    19


<210> 13
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 13
caaatctctc ggcttctac                                                    19


<210> 14
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 14
cgtgttgtgt taagcgtgt                                                    19


<210> 15
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =

synthetic construct
<400> 15
ccgcggaaaa ggaaccact                                                         19


<210> 16
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 16
ctcctggcag cccagtgag                                                         19


<210> 17
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 17
ctcctcccct cgatgcttc                                                         19


<210> 18
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 18
cagttaccct ctgcagatc                                                         19


<210> 19
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 19
ctatttcctc tgcagataa                                                         19


<210> 20
<211> 22
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct

<221> misc_feature
<222> 11-16
<223> N = a, g, c, or t (u)
<400> 20
ccgactcgag nnnnnnatgt gg                                                     22


<210> 21
<211> 19
<212> DNA

```
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 21
ctgaaacatc gcactatcc                                                    19


<210> 22
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 22
ccaaactttg aaatgtttg                                                    19


<210> 23
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 23
ctgccaccaa ttcctgctt                                                    19


<210> 24
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 24
cataagcaaa ggccatctt                                                    19


<210> 25
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 25
ggaagcaatt caagatctg                                                    19


<210> 26
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct

<400> 26
cttcagattg cagcaatgt                                                    19


<210> 27
<211> 19
<212> DNA
```

```
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 27
gaattaatga aattgggag                                              19


<210> 28
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct

<400> 28
actgttagtt tccataggc                                              19


<210> 29
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 29
caaggttgat tattttaga                                              19


<210> 30
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 30
agtactagtt catgttttc                                              19


<210> 31
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 31
tagtactcct taagaacac                                              19


<210> 32
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 32
ctaacattcg atctcattc                                              19


<210> 33
<211> 19
<212> DNA
```

```
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 33
gcggacgggc tgtccccgc                                                    19


<210> 34
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 34
ggaaggactt ggcgcgccc                                                    19


<210> 35
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 35
aactaaagcc aagggtatc                                                    19


<210> 36
<211> 18
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 36
ataacacaga gagacaga                                                     18


<210> 37
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 37
caactcatgc taacgcagc                                                    19


<210> 38
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 38
ggaagctgga gagaatcgc                                                    19


<210> 39
<211> 19
<212> DNA
```

```
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 39
gacatggagt cccaggagc                                              19

<210> 40
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct
<400> 40
aggccctgaa ggaggagcc                                              19
```

(72) Inventors: LASKEN, Roger, S.; 364 Central Avenue,
First Floor, New Haven, CT 06515 (US). EGHOLM,
Michael; 8 Dogwood Court, Woodbridge, CT 06525 (US).
ALSMADI, Osama, A.; 310 Glenwood Drive, Guilford,
CT 06437 (US). BORNARTH, Carole; 99 Mohawk
Drive, Wallingford, CT 06492 (US). WISNIEWSKI,
Michele; 8302 Town Walk Drive, Hamden, CT 06518
(US). HOSONO, Seiyu; 140 New Street, Apt. 2315,
Mamaroneck, NY 10543 (US). RAGHUNATHAN, Aru-
mugham; 80 Brentwood Drive, Cheshire, CT 06410 (US).

(54) Title: NUCLEIC ACID AMPLIFICATION

(57) Abstract: Disclosed are compositions and a method for amplification of nucleic acid sequences of interest. The method is based
on strand displacement replication of the nucleic acid sequences by primers. The disclosed method is a form of multiple displacement
amplification (MDA) useful for amplifying genomic nucleic acid samples and other nucleic acid samples of high complexity. The
disclosed method can be used to amplify such highly complex nucleic acid samples using only one or a limited number of primers.
It has been discovered that one or a small number of primers can effectively amplify whole genomes an other nucleic acid samples
of high sequence complexity. The primers are specially selected or designed to be able to prime and efficiently amplify the broad
range of sequences present in highly complex nucleic acid samples despite the limited amount of primer sequence represented in
the primers. It has been discovered that generation of high molecular weight artifacts, in an isothermal amplification procedure,
is substantially reduced or eliminated while still allowing the desired amplification of input DNA by carrying out the reaction at a
higher temperature and, optionally, in the presence of one or more additives. It has also bee discovered that amplification reactions
can produce amplification products of high quality, such as low amplification bias, if performed on an amount of nucleic acid at or
over a threshold amount and/or on nucleic acids at or below a threshold concentration.

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7)  :  C12Q 1/68; C12P 19/34, C07H 21/04

US CL  :  435/6, 91.2; 536/24.33

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2; 536/24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X --- Y | US 6,124,120 (LIZARDI) 26 September 2000 (26.09.2000) entire document, especially column 26, line 20-67, col. 18, line 32-46, col. 19, line 21-45. | 1-2, 7-8, 12, 14, 17, 50, 54-55 ---------- 3-11, 13-16, 18-49, 51-53, 56-185 |
| X --- Y | US 6,323,009 (LASKEN et al.) 27 November 2001 (26.11.2001) entire document, especially column 5, line 12-64. | 147-184, 186-188, 190 ---------- 17-146, 189, 191-285 |
| Y | US 6,479,235 (SCHUMM et al) 12 November 2002 (12.11.2002), entire document, especially columnb4, line 22-65 | 1-285 |

☐ Further documents are listed in the continuation of Box C.     ☐ See patent family annex.

| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
|---|---|---|---|
| "A" | document defining the general state of the art which is not considered to be of particular relevance | | |
| "E" | earlier application or patent published on or after the international filing date | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | "&" | document member of the same patent family |

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 18 March 2005 (18.03.2005) | 04 APR 2005 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US<br>Commissioner of Patents<br>P.O. Box 1450<br>Alexandria, Virginia 22313-1450 | Authorized officer<br><br>Suryaprabha Chunduru |
| Facsimile No. (703) 305-3230 | Telephone No. 703-308-0196 |

Form PCT/ISA/210 (second sheet) (July 1998)

# INTERNATIONAL SEARCH REPORT

**Continuation of B. FIELDS SEARCHED Item 3:**
Biosis, Embase, Medline, Lifesci, Caplus and EAST databases
search terms: whole genome amplification, repeat sequence, strand displacement or isothermal amplification